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METHODS OF ANALYSIS

FOR

WATERS, ORGANIC MATTER, AND POND BOTTOM SOILS

USED IN FISHERIES RESEARCH

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1969 Revision
by G. N. Greene
R. T. Lovell

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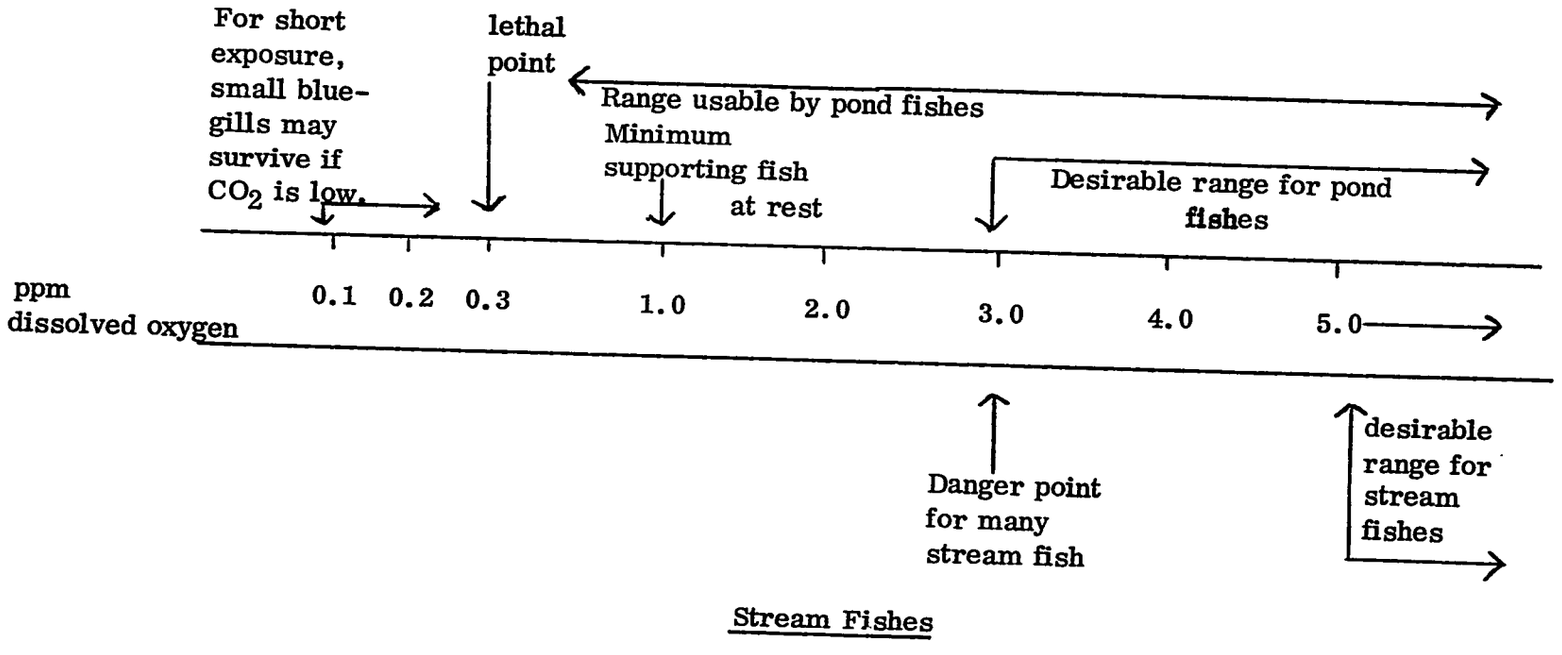


WINKLER METHOD FOR DETERMINATION OF DISSOLVED OXYGEN

This method of oxygen analysis determines the free dissolved O_2 in water. It is based on the following reactions:

1. $MnSO_4 + 2\ NaOH \dashrightarrow Mn(OH)_2 + Na_2SO_4$

2. $Mn(OH)_2 + O \dashrightarrow H_2MnO_3$ (brown precipitate)

3. $H_2MnO_3 + 2\ H_2SO_4 + 2\ KI \dashrightarrow MnSO_4 + 3\ HOH + K_2SO_4 + I_2$

The released I_2 gives the solution a yellow to brownish color, and the intensity of the color is proportional to the O_2 content of the original water sample. The amount of I_2 is determined by titration with sodium thiosulfate:

4. $I_2 + 2\ Na_2S_2O_3 \dashrightarrow Na_2S_4O_6 + 2\ NaI$

The end point is determined by the use of starch indicator, which gives a blue color with free I_2 and no color with NaI. The disappearance of the blue color marks the end of the reaction.

If the titrated solution is allowed to stand for several minutes, subsequently the blue color returns due to absorption of additional O_2 from the air and the release of I_2 from the HI present in the acid solution:

5. $4\ HI + O_2 \dashrightarrow 2\ I_2 + 2\ H_2O$

Also, presence of Fe (ic) or NO_2 may cause subsequent release of free I_2 from KI or NaI. Consequently, the sample should not be again titrated after the original end-point has been reached.

Materials Interfering with the Accuracy of the Method

Certain materials when present in waters interfere with the accuracy of the Winkler method.

I. Turbidity: Muddy waters and those with heavy suspended organic matter should be cleared as follows:

To a 1-liter water sample, add 10 ml of 10% potassium aluminum sulfate. Then add 1 ml 35% NaOH. Stopper and rotate. Allow the precipitate to settle and analyze the clear supernatant liquid. (Avoid excess of NaOH because at pH above 11, organic matter absorbs O_2 rapidly.)

For activated sludges and heavy organic matter, clear as follows:

Add 10 ml copper-sulfamic acid to 1-liter bottle. Add water sample by siphoning. Mix, and allow to settle.

Copper-sulfamic acid: Dissolve 16 gm sulfamic acid in 200 ml distilled water. Dissolve 25 gm $CuSO_4 \cdot 5H_2O$ in 250 ml water. Mix and add 12 ml glacial acetic acid.

II. Materials causing high results: Nitrites and ferric iron tend to cause high results because they react with KI to release I_2.

Nitrites are present in most waters containing fish. For more accurate results, the sulfamic acid modification or the potassium permanganate modification may be used. The reactions are as follows:

$$HNO_2 + NH_2 SO_2OH \dashrightarrow H_2SO_4 + N_2 + H_2O$$

$$2KMnO_4 + 5NaNO_2 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 5NaNO_3 + 3H_2O$$

Ferric iron: Except at concentrations in excess of 5 ppm, ferric iron causes little error. High concentrations of ferric iron are normally present only in strongly acid waters. The addition of 1 ml of 40% $KF \cdot 2H_2O$ is sufficient to prevent interference up to 200 ppm Fe^{+++}. It should be added prior to starting the standard Winkler determination. This treatment is principally necessary in analyzing water from streams receiving acid mine wastes.

III. Materials causing low results: Ferrous iron, sulfites and organic matter cause low results by removing the liberated I_2 from the treated sample. Organic matter may absorb I_2 especially at high alkalinities, and also causes loss of dissolved O_2 prior to the analysis.

Ferrous iron: A concentration of 1 ppm, Fe^{++} causes the loss of 0.14 ppm O_2. Since ferrous iron is principally present in waters very low or lacking in dissolved oxygen (D.O.), it seldom requires removal in well-oxygenated waters. When present in excess of 1 ppm, the water sample should be pretreated with the potassium permanganate modification:

$$10\ FeSO_4 + 2KMnO_4 + 8H_2SO_4 \longrightarrow 5\ Fe_2(SO_4)_3 + 2\ MnSO_4 + K_2SO_4 + 8H_2($$

Sulfites and organic matter: These materials found in paper mill effluents make analysis of oxygen difficult. The alkaline hypochlorite modification has been proposed (See APHA Standard Methods) but the reagents are difficult to prepare and must be restandardized weekly. In river waters receiving such effluents, the sulfamic acid modification gives reasonably accurate results. When organic matter is present in sufficient amount to make the water murky, the water should be treated with Cu-sulfamic acid as explained above. The analysis of the clear supernatant liquid should then proceed promptly and rapidly.

Preliminary Tests to Determine Presence of Interfering Materials

Reagents:

1. 5% KI: Dissolve 5 gm KI in 100 ml distilled water.
2. HCl: Concentrated
3. Iodine stock: Dissolve 6 gm KI + 4 gm I_2 in 100 ml distilled water.

4. Dilute I_2 solution: Add 1 drop stock Iodine to 100 ml distilled water. Store in brown glass-stoppered bottle not over 3 days.
5. Potassium thiocyanate 5%: Dissolve 5 gm in 100 ml distilled water.
6. Potassium ferrocyanide 5%: Aqueous solution, as above.
7. Potassium ferricyanide 5%: Aqueous solution, as above.
8. Sulfanilic acid solution: Dilute 30 ml glacial acetic acid to 100 ml with distilled water. Add 1 gm sulfanilic acid.
9. Alpha-Naphthylamine acetate: Dilute 30 ml glacial acetic acid to 100 ml with distilled water. Add 0.5 gm alpha-naphthylamine, shake and filter through cotton. Store in a brown bottle in the dark.

Procedure:

1. Collect about 50 ml of the water sample in an open beaker.
2. Filter, if necessary, through filter paper or cotton.
3. To a 10 ml water sample, add 1 ml of 5% KI and 1 ml concentrated HCl. Mix. A brown to yellow color indicates presence of materials causing high results. See Section A following.
4. To a 10 ml sample add 2 drops starch and then drop by drop add dilute I_2, shaking after each drop. Record the number of drops required for a permanent blue color.

   In another flask, place 10 ml distilled water and add 2 drops starch solution. Add drop by drop of dilute I_2 solution shaking after each drop. Record the number of drops required for a permanent blue color.

   If the drops required for the water sample are less than that for the distilled water, this indicates the presence of materials absorbing I_2 and causing low results: e.g., ferrous iron, sulfites, organic matter and/or similar substances. See Section B following.

A. Materials causing high results (releasing I_2):

a. Nitrites:

Test: Acidify a 50-ml water sample with a drop of HCl, add 2 ml of sulphanilic acid and 2 ml naphthylamine hydrochloride. Nitrites cause a pink coloration.

b. Ferric iron:

Test: Acidify a 10-ml water sample with 2 drops conc. HCl. Add 0.5 ml 5% K thiocyanate. A pink to red color indicates ferric ion; 10 ppm Fe^{+++} gives a very slight pink and 15 ppm a light red.

Test: To a neutral or only slightly acid water sample add several drops K ferrocyanide. The color varies from green to blue with increasing amounts of Fe^{+++}; 1 ppm gives a faint blue-green on standing, 5 ppm gives a light blue within several minutes and 10 ppm gives a deep blue. The reaction is:

$$4\ FeCl_3 + 3\ K_4\ Fe(CN)_6 \rightarrow Fe_4\ (FeC_6N_6) + 12\ KCl.$$

Prussian blue

This test is sensitive to 0.002 mg ferric ion. Ferrous iron may give a white precipitate.

B. Materials causing low results (absorbing I_2):

a. Ferrous iron:

Test: To a 10-ml water sample add a few drops of potassium ferricyanide solution. A blue precipitate indicates the presence of ferrous iron (Fe^{++}). The reaction is:

$$3\ FeSO_4 + 2\ K_3\ Fe(CN)_6 \rightarrow Fe_3(FeC_6N_6) + 3\ K_2\ SO_4$$

Turnhill blue

This blue precipitate is insoluble in HCl. A solution of 1 ppm Fe^{++} gives a light green color, 10 ppm a blue-green color and 50 ppm gives a deep blue. Ferric iron may give a brown color.

Modifications of the Winkler Method

For exceptional waters containing considerable amounts of ferrous iron, the Rideal-Stewart modification of the Winkler method may be used. This involves oxidation of the ferrous iron to ferric iron with potassium permanganate.

For most waters, nitrite (NO_2^-) is the principal interfering salt. Sulfamic acid pretreatment reduces the error from this interference:

$$NH_2SO_2OH + HNO_2 \rightarrow H_2SO_4 + N_2 + H_2O$$

Since the sulfamic acid is stable in sulfuric acid and easy to use, this modification is used in all waters containing fish.

Sodium azide (NaN_3) may also be used to eliminate interference by nitrites:

$$NaN_3 + H_2SO_4 + HNO_2 \rightarrow N_2 + H_2O + N_2O + NaHSO_4$$

This method is not given although it is as effective as the sulfamic acid modification.

The Iodine Difference Method of Ohle may be used in problem waters or when great accuracy is desired. This corrects both for the presence of materials causing high results by releasing iodine from KI and those causing low results by absorption of free iodine.

Reagents:

For Rideal-Stewart Modification:

1. Potassium permanganate solution: Dissolve 3.16 gm potassium permanganate in distilled water and dilute to 500 ml. Transfer to a clean brown bottle and keep in the dark.

2. Potassium oxalate solution: Dissolve 10 gm of potassium oxalate in water and dilute to 500 ml.

For Sulfamic Acid Pretreatment of Water:

1. Sulfamic acid solution: Add 20 ml concentrated sulfuric acid to 80 ml water. Add 4 gm sulfamic acid and shake until dissolved. Store in brown bottle with glass stopper.

For Standard Winkler Method:

1. Manganous sulfate solution: Dissolve 240 gm of manganous sulfate in water and dilute to 500 ml.
2. Alkaline potassium iodide solution: Dissolve 250 gm of sodium hydroxide and 75 gm of potassium iodide in water and dilute to 500 ml. Use a flask with a rubber stopper for storage.
3. Standard 0.025 N sodium thiosulfate solution: In reaction 4, page 2, 2 molecules of sodium thiosulfate reacted with 2 iodide equivalents. Therefore, an N/1 solution of sodium thiosulfate contains the molecular weight of this compound in gm per liter of solution. The salt used is $Na_2S_2O_3 \cdot 5H_2O$, with a molecular weight of 248.19. A 0.025 N solution then contains:

$$248.19 \times 0.025 = 6.2048 \text{ gm per liter of solution.}$$

Weigh accurately 6.205 gm of chemically pure recrystallized sodium thiosulfate. Place in a 1-liter glass-stoppered volumetric flask and dilute to 1 liter with freshly boiled and cooled distilled water. Add several drops chloroform as a preservative, label, and stopper tightly. Keep this reagent in the dark.

Each ml of 0.025 N sodium thiosulfate is equivalent to 0.2 mg of oxygen or to 0.1400 ml of oxygen at 0 C and 760 mm pressure.

As this reagent is not permanent, it should be restandardized at 30-day intervals against a 0.025 N solution of potassium dichromate.

4. Standard 0.025 N potassium dichromate solution: This salt makes a permanent standard and should be prepared carefully. It reacts with KI in acid solution to liberate a definite amount of I_2 as follows:

$$6\ KI + K_2Cr_2O_7 + 14\ HCl \rightarrow 8\ KCl + CrCl_3 + 7\ H_2O + 3\ I_2$$

The liberated iodine is then titrated with the thiosulfate solution. Since in the above reaction one molecule of $K_2Cr_2O_7$ reacts with 6 iodine equivalents, a normal solution of potassium dichromate equals the molecular weight in grams divided by 6. The $K_2Cr_2O_7$ contains no water of crystallization and has a molecular weight of 294.2; a 0.025 N solution of this salt then contains:

$$\frac{294.2}{6} \times 0.025 = 1.2258 \text{ gm per liter}$$

Weight accurately 0.6129 gm of pure crystalline $K_2Cr_2O_7$ and dilute to 500 ml in a glass-stoppered volumetric flask with freshly boiled and cooled distilled water.

5. 25% potassium iodide solution: Dissolve 25 gm KI in 100 ml freshly boiled and cooled distilled water.

6. Starch indicator: Add 2 gm soluble starch to 100 ml water. Heat until transparent. Add 0.5 ml formalin for preservative.

Or use:

Modified starch glycerite:

1. Add 3 gm powdered soluble starch to 100 ml glycerin.
2. Heat to 190 C.
3. Cool to room temperature. If solution gels with age, reheat to 190 C and add glycerin.

Starch indicator is stable for about 30 days, while the modified starch glycerite keeps indefinitely.

Standardization of Approximately 0.025 N Sodium Thiosulfate:

1. Place in a beaker 100 ml of freshly boiled and cooled distilled water.
2. Add 3 ml of a 25% solution of KI.
3. Add from a burette 50 ml of the standard 0.025 N potassium dichromate.
4. Add 10 ml concentrated HCl.
5. Titrate the liberated I_2 with the standard 0.025 N sodium thiosulfate solution. When the yellow color has almost disappeared, add about 2 ml starch indicator and continue the titration until the blue color just disappears. The final color may be a light green due to chromic salts.
6. The factor for the 0.025 N thiosulfate equals:

$$\frac{\text{ml standard dichromate}}{\text{ml standard thiosulfate}} = \frac{50}{X}$$

For example, if the titration requires 48.8 ml of approximately 0.025 N thiosulfate, the factor $= \frac{50}{48.8} = 1.024$

Collection of Sample

Collect the sample in a narrow-necked glass-stoppered bottle of 250 to 300 ml capacity (standard B.O.D. bottle) by means of an apparatus designed to avoid the entrapment or absorption of any oxygen from the atmosphere. Note the temperature.

Determination of Dissolved Oxygen

Removal of Ferrous Iron and Nitrites: Follow Modification I only if iron or other interfering materials prevent the use of Modification II.

Modification I. Rideal-Stewart Modification

1. Remove the stopper from the bottle and add 0.7 ml of concentrated sulfuric acid and then 1 ml of the potassium permanganate solution. Introduce these and all other reagents by pipette under the surface of the liquid. Insert the stopper and mix by inverting the bottle several times.

2. If a noticeable excess of potassium permanganate is not present after 5 minutes, again add 1 ml of the permanganate solution; if this is still insufficient, use a stronger permanganate solution, but avoid a large excess.

3. After 20 minutes destroy the excess permanganate by adding 1 ml of the potassium oxalate solution, re-stopper the bottle at once, and mix its contents. Let stand several minutes. If the pink color persists, add an additional 1 ml of potassium oxalate, re-stopper, and mix. Repeat until solution is free of pink color, but avoid adding excess oxalate as this causes low results. (Continue with Standard Winkler Method.)

Modification II. Sulfamic Acid Modification

Use this modification for most pond waters.

1. Remove the stopper and add 1 ml of the sulfamic acid solution. Replace stopper and mix by inverting bottle. After 30 seconds, continue with the Standard Winkler Method of analysis.

Standard Winkler Method

1. Add 1 ml of the manganous sulfate solution and 3 ml of the alkaline

potassium iodide solution. Mix by rapid inversion for 30 seconds. Allow the precipitate to settle. Reshake and allow to settle. (For sea water, alternately shake and allow to settle for a 10-minute period.)

2. Add 1 ml of concentrated sulfuric acid and mix by shaking. If water is high in iron or organic matter, continue promptly with steps 3 and 4.

3. Measure 200 ml of the contents of the bottle into a graduated cylinder or pipette. Discard the remainder of the sample and carefully pour the measured sample back into the sample bottle or into an Erlenmeyer flask with the minimum of aeration.

4. Titrate with 0.025 N thiosulfate until the sample becomes a faint yellow. Add a few drops of the starch indicator and continue the titration until the blue color disappears.

5. Report results as parts per million. Each ml of standard 0.025 N sodium thiosulfate equals 1 ppm O_2. For example, if the titration required 4.5 ml of 0.025 N thiosulfate, the sample contained 4.5 ppm O_2.

If the thiosulfate had a factor of 1.024, each ml = 1.024 ppm O_2 and the above sample contained:

$$4.5 \times 1.024 = 4.6 \text{ ppm } O_2.$$

MODIFIED WINKLER FOR DISSOLVED OXYGEN BY THE IODINE DIFFERENCE METHOD (Ohle)

Reagents:

1. Iodine Stock Solution

   Dissolve 5 gm iodine plus 100 gm KI in 114 ml water. One ml of this solution contains about 44 mg I_2 and 0.88 gm KI.

2. Iodine Reagent

   Dissolve 35 gm NaCl in 100 ml distilled water; add 10 ml of the above Iodine Stock Solution. One ml contains about 4 mg I_2 and 80 mg KI.

3. Alkaline - KI

   Prepared as described on page 8.

4. Manganous Sulfate

   Prepared as described on page 8.

5. Concentrated H_2SO_4

6. Starch Solution

   Boil 1 gm soluble starch in 100 ml distilled water; cool, add 0.5% formalin.

7. Sodium Thiosulfate

   Prepared as described on page 8.

Procedure:

1. Collect 2 duplicate water samples from exactly the same depth of water using BOD bottles marked A and B. Samples must be taken by water sampler or bottle train in such a way that the samples are not contaminated by contact with air.

2. To each of the 2 samples, A and B, add 2 ml of Iodine reagent. If the free iodine disappears, add an additional 2 ml to each sample. Cover, or keep out of light. The duration of treatment with the iodine reagent must be the same for each of the two samples.

3. To bottle A, add:

   1 ml $MnSO_4$

   3 ml alkaline-KI,

   Stopper and shake briskly for 10 to 15 seconds. Then add 1.5 ml concentrated H_2SO_4 before the precipitate begins to settle. Let stand 10 minutes. Titrate 200 ml with thiosulfate, using starch indicator near the end point.

4. To bottle B, add:

   3 ml alkaline-KI,

   Stopper and shake for 10 to 15 seconds. Add 1.5 ml concentrated H_2SO_4 before the precipitate settles. Let stand 10 minutes. Titrate 200 ml with thiosulfate as above.

5. The difference in titre of the 2 samples (A and B) is equivalent to the dissolved oxygen in the water sample plus that added in the reagents. The iodine equivalent to dissolved oxygen is liberated in bottle A only. The titre for the second bottle (B) corrects for interfering substances liberating and/or absorbing iodine if the reaction time for the two is the same.

6. For most accurate determination of O_2, the proportionate volumes of reagents must be subtracted from the volume titrated and the dissolved

oxygen in the reagents must be subtracted from the results of the titration. Correction for volumes of reagents can be obtained by titrating 210 ml instead of 200 ml.

Modified from: Ohle, Waldemar. 1953. Die Chemishe und die Electrochemische Bestimmung des Molekular Gelosten Sauerstoffs der Binnengewasser. Int. Verein. fier Theoretische und Angew. Limnologie Mitteilungen. No. 3:44 pp.

PORTABLE POLAROGRAPHIC OXYGEN METER

Principles

The system consists of a gold electrode and a silver-silver oxide reference electrode. When a suitable polarizing voltage is applied across the cell, molecular oxygen is reduced to form OH^- at the gold electrode

$$4\,e^- + O_2 + 2\,HOH \text{ --- } 4\,OH^-$$

A current then flows to the silver electrode and the OH ions react with the silver to form Ag_2O

$$2\,Ag + 2\,OH^- \text{ --- } Ag_2O + HOH + 2\,e^-$$

The Ag_2O appears as black or brown coating on the silver, and acts to form a half-cell which completes the circuit with the gold electrode. The amount of current flowing between the two electrodes depends upon the amount of O_2 reaching the gold electrode.

Since organic materials and metals accumulate upon a bare gold electrode and reduce its sensitivity, it is necessary to enclose the electrodes (bathed in 0.5N K Cl) within a teflon membrane. Molecular oxygen can diffuse through the membrane from the water, but ions cannot. Membranes of a thickness from 0.5 to 1 mil are used. The current flow will be approximately 1/1000 of that which would flow between the bare electrodes if no membrane were used. Since the oxygen must pass through the membrane and the O_2 inside the membrane is effectively zero, the apparatus measures the oxygen tension.

The Winkler analysis for dissolved oxygen (DO) determines oxygen in ppm, whereas the oxygen meter measures oxygen tension.

Parts per million DO is the weight in milligram of dissolved oxygen per liter of water. Oxygen tension is the pressure of oxygen above a water surface required to maintain a certain weight of oxygen gas in solution when the two are at equilibrium --- that is when the same numbers of molecular of oxygen are leaving the water as are entering from the air above per unit of time. This water is said to be "saturated" with oxygen.

Temperature, however, has an important effect upon the ppm dissolved oxygen in water at saturation. The higher the water temperature, the higher the partial pressure required to maintain, for example, 9 ppm dissolved oxygen. In pure water at 20 C and 760 air pressure water is "saturated" at 9.2 ppm oxygen. However, if air pressure remains the same and water temperature rises to 25 C, the water with 9.2 ppm DO is "supersaturated" and will lose oxygen to the air until 8.4 ppm is reached, where the water is "saturated" at the new temperature. However, since the air pressure did not change, and since oxygen is 20.92% of air, the oxygen tension was 760 x 0.2092, or 160 mm in both cases. The oxygen meter consequently gives the same reading in saturated water at both temperatures.

In addition, the salt content of water has an effect similar to that of temperature. The higher the salt content, the greater the oxygen tension required to maintain a certain weight of oxygen in solution. At 760 mm pressure and 25 C, pure water holds 9.2 ppm oxygen at saturation, while seawater (3.5% salt) holds only 7.4 ppm. The oxygen meter will read approximately the same figure for both because the oxygen tension is the same.

It is thus evident that the oxygen meter must be calibrated for temperature and salinity to convert its readings into ppm.

Operation

These directions are written for use with a YSI model 51 oxygen meter but can be used with appropriate modifications with other machines. For best results read the instruction manual for the instrument to know how to use it properly.

Preparation of probe:

1. Remove guard, old membrane, and "O" ring and rinse electrodes with distilled water or K Cl solution.
2. Fill hole in probe with K Cl and form a large drop over gold electrode.
3. Stretch membrane over electrodes, avoiding bubbles, and fasten down with "O" ring. While handling, keep membrane clean and free of fingerprints.
4. Trim excess membrane and replace guard. Probe is ready for operation.

Procedure:

1. Turn selector switch to red line and adjust needle to the line with slotted control knob. If you cannot adjust needle to the line the batteries need replacing (see instrument instruction manual).
2. Turn selector to zero and adjust needle to zero.
3. Lower probe into water, turn selector to temperature and read temperature. Allow 30 seconds to 1 minute for equilibration.
4. Turn selector to read, gently move probe or otherwise cause water to flow across membrane, and read O_2. Low readings will result if there is no flow across the membrane.

Calibration:

The meter may be calibrated against a Winkler determination of dissolved oxygen in the water or for fresh waters it may be calibrated in air. For greatest accuracy, calibration should be done by the Winkler analysis in waters of the same salinity and at the same temperature (± 3 C) as the measurements to be made. Where this is impossible, corrections can be made but the accuracy is lowered.

To calibrate in air:

1. Place probe in damp calibration chamber and place in water to be measured.
2. Allow temperature to equilibrate (3 to 5 minutes).
3. Read temperature and determine calibration value (saturation) for the temperature and atmospheric pressure from calibration table.
4. Turn selector to read and adjust meter to calibration value.

To calibrate against Winkler determination:

1. Determine oxygen by Winkler method.
2. Insert probe, turn selector to read and adjust meter to value determined in step 1.

Notes

Accuracy:

The accuracy is greatest (± 0.3 ppm) when calibration and measurements are made at the same temperature. When the temperature difference reaches 10 C, the error may be as great as 2 ppm. When the O_2 is below 1 ppm, the meter readings are only approximate. It is also inaccurate in supersaturated waters.

Calibration Table

Solubility of oxygen in water (saturated with air) in ppm at various temperatures and pressures

P mm	775	760	750	725	700	675	650	625
P inches	30.51	29.92	29.53	28.54	27.56	26.57	25.59	24.61
°C	ppm:							
0	14.9	14.6	14.4	13.9	13.5	12.9	12.5	12.0
1	14.5	14.2	14.1	13.6	13.1	12.6	12.2	11.7
2	14.1	13.9	13.7	13.2	12.9	12.3	11.8	11.4
3	13.8	13.5	13.3	12.9	12.4	12.0	11.5	11.1
4	13.4	13.2	13.0	12.5	12.1	11.7	11.2	10.8
5	13.1	12.8	12.6	12.2	11.8	11.4	10.9	10.5
6	12.7	12.5	12.3	11.9	11.5	11.1	10.7	10.3
7	12.4	12.2	12.0	11.6	11.2	10.8	10.4	10.0
8	12.1	11.9	11.7	11.3	10.9	10.5	10.1	9.8
9	11.8	11.6	11.5	11.1	10.7	10.3	9.9	9.5
10	11.6	11.3	11.2	10.8	10.4	10.1	9.7	9.3
11	11.3	11.1	10.9	10.6	10.2	9.8	9.5	9.1
12	11.1	10.8	10.7	10.3	10.0	9.6	9.2	8.9
13	10.8	10.6	10.5	10.1	9.8	9.4	9.1	8.7
14	10.6	10.4	10.2	9.9	9.5	9.2	8.9	8.5
15	10.4	10.2	10.0	9.7	9.3	9.0	8.7	8.3
16	10.1	9.9	9.8	9.5	9.1	8.8	8.5	8.1
17	9.9	9.7	9.6	9.3	9.0	8.6	8.3	8.0
18	9.7	9.5	9.4	9.1	8.8	8.4	8.1	7.8
19	9.5	9.3	9.2	8.9	8.6	8.3	8.0	7.6
20	9.3	9.2	9.1	8.7	8.4	8.1	7.8	7.5
21	9.2	9.0	8.9	8.6	8.3	8.0	7.7	7.4
22	9.0	8.8	8.7	8.4	8.1	7.8	7.5	7.2
23	8.8	8.7	8.5	8.2	8.0	7.7	7.4	7.1
24	8.7	8.5	8.4	8.1	7.8	7.5	7.2	7.0
25	8.5	8.4	8.3	8.0	7.7	7.4	7.1	6.8
26	8.4	8.2	8.1	7.8	7.6	7.3	7.0	6.7
27	8.2	8.1	8.0	7.7	7.4	7.1	6.9	6.6
28	8.1	7.9	7.8	7.6	7.3	7.0	6.7	6.5
29	7.9	7.8	7.7	7.4	7.2	6.9	6.6	6.4
30	7.8	7.7	7.6	7.3	7.0	6.8	6.5	6.2
31	7.7	7.5	7.4	7.2	6.9	6.7	6.4	6.1
32	7.6	7.4	7.3	7.0	6.8	6.6	6.3	6.0
33	7.4	7.3	7.2	6.9	6.7	6.4	6.2	5.9
34	7.3	7.2	7.1	6.8	6.6	6.3	6.1	5.8
35	7.2	7.1	7.0	6.7	6.5	6.2	6.0	5.7
36	7.1	7.0	6.9	6.6	6.4	6.1	5.9	5.6
37	7.0	6.8	6.7	6.5	6.3	6.0	5.8	5.6
38	6.9	6.7	6.6	6.4	6.2	5.9	5.7	5.5
39	6.8	6.6	6.5	6.3	6.1	5.8	5.6	5.4
40	6.7	6.5	6.4	6.2	6.0	5.7	5.5	5.3

Dissolved salts:

Since the meter measures oxygen tension and not absolute quantity and since the solubility of gases decreases with increased dissolved salts, the meter will give high readings as ppm DO in saline waters if calibrated in air without correcting for salt content. (A change from O (distilled water) to 3.5 per cent salts (sea water) decreases O_2 solubility by about 20 per cent so the meter will read about 20 per cent high.) The dissolved salts in most fresh waters do not cause a significant change from distilled water. Calibration against a Winkler determination eliminates this source of error in saline waters.

ACIDITY AND ALKALINITY OF WATER

Determination of Free CO_2, and HCO_3^-, CO_3^{--} and OH^- Alkalinities

The titration method is described here for these determinations. A nomographic method of determination may be found in APHA Standard Methods.

The approximate relationships of CO_2, and HCO_3^-, CO_3^{--}, and OH^- alkalinities are shown on the accompanying chart.

Separation of the various forms of CO_2 is based on the assumption that the end point of phenolphthalein (approximately 8.3), gives a sufficiently satisfactory division between carbonates and bicarbonates for most biological purposes. In more alkaline waters from .3 to 10.5, there may be a mixture of HCO_3^- and CO_3^{--} but the bicarbonate decreases sharply as the pH value approaches 10.4. At higher pH values relatively insignificant amounts of bicarbonate are present.* In waters more acid than 8.3, practically no carbonate is present, but only varying mixtures of HCO_3^- and CO_2.

In waters that are exposed to the air and are more acid than pH 5.5, acidity is assumed to be due to stronger acids than CO_2. When at equilibrium with air at atmospheric pressure of 760 mm, water contains only approximately 0.4 ppm free CO_2 and can not make the water more acid than 5.5. However, water from deep wells, from springs or the deeper waters of lakes or ponds where CO_2 gas is released by decomposition, are exposed to much higher partial pressures of the gas and may contain up to 200 or more ppm of free CO_2, with

---

*Except in waters which contain high concentrations of Na and K salts.

correspondingly lower pH values. Upon exposure to air, the free CO_2 is rapidly lost until equilibrium with the partial pressure of CO_2 in the atmosphere is reached. CO_2 can be rapidly removed from water by heating the water to boiling.

Titration with either Na_2CO_3 or NaOH to the phenolphthalein end point allows estimation of free CO_2 in water.

Principles

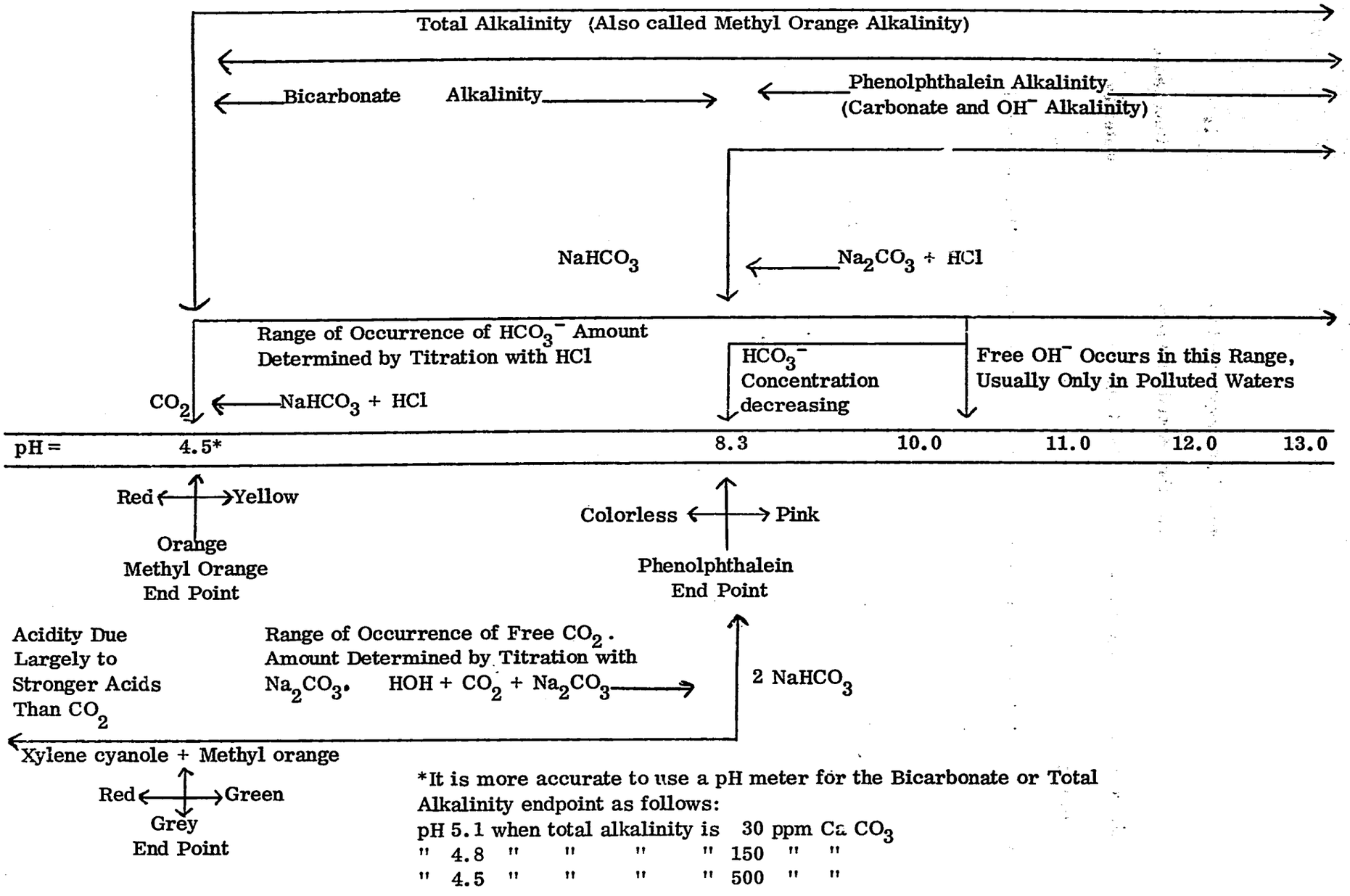
1. Water sample acid to phenolphthalein and alkaline to methyl orange:

   Contains free CO_2 and bicarbonates.

   a. Determine free CO_2 by titration with Na_2CO_3 with phenolphthalein indicator.

$$CO_2 + HOH + Na_2CO_3 \longrightarrow 2\ NaHCO_3$$

RELATIONSHIP AND DETERMINATION OF CO_2, HCO_3^-, CO_3^{--}, AND OH^- IN NATURAL WATERS



*It is more accurate to use a pH meter for the Bicarbonate or Total Alkalinity endpoint as follows:

pH 5.1 when total alkalinity is 30 ppm $CaCO_3$
" 4.8 " " " " 150 " "
" 4.5 " " " " 500 " "

The equivalent weight of Na_2CO_3 in this reaction is the molecular weight divided by two.

b. Determine the HCO_3^- in a fresh sample by titration with HCl, with methyl orange indicator.

$$HCl + NaHCO_3 \rightarrow NaCl + HOH + CO_2$$

2. Water sample alkaline to phenolphthalein (pink color):

Contains CO_3^{--}, HCO_3^- or possibly OH^-

a. Determine phenolphthalein alkalinity by titration with standard HCl to phenolphthalein end point (pH = 8.3).

$$Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl$$

The equivalent weight of Na_2CO_3 is the molecular weight, since 1 molecule combines with 1 equivalent of HCl.

b. Using the colorless solution from the above titration, determine the bicarbonate alkalinity by titration with standard acid to the methyl orange or the methyl orange - xylene cyanole end point (pH = 4.5).

$$NaHCO_3 + HCl \rightarrow NaCl + HOH + CO_2$$

This bicarbonate is a mixture of the bicarbonate originally present and that formed in the preceding titration from the carbonate, if present. The latter will require the same volume of acid as was used in the original titration under Section a. If this amount is subtracted from the volume used in the second titration, the difference is that required by the bicarbonate originally present.

Interpretation:

A. Total alkalinity is due not to carbonates but to OH^- when a sample has phenolphathalein alkalinity but no bicarbonate alkalinity, because the titration of carbonate to the phenolphthalein end point would have produced bicarbonate.

B. Total alkalinity is due to only HCO_3^- if there is bicarbonate alkalinity but no phenolphthalein alkalinity.

C. Total alkalinity is due to only CO_3^{--} if the bicarbonate alkalinity equals the phenolphthalein alkalinity.

D. Total alkalinity is due to OH^- and CO_3^{--} when phenolphthalein alkalinity is greater than the bicarbonate alkalinity.

Reagents:

1. Standard 0.0454N Na_2CO_3 solution:

The molecular weight of sodium carbonate is 106, and since it contains 2 equivalents, a 1.0 N solution contains $\frac{106.0}{2}$ = 53.0 gm per liter or 26.5 gm per 500 ml.

Preparation of Stock Standard Solution of Na_2CO_3:

a. Heat a clean 100 ml evaporating dish to 200 C in an oven for 30 minutes. (Or heat at 120 C for 10 hours.) Cool in a desiccator. Remove and weight accurately.

b. Add approximately 30 gm of C.P. reagent-grade Na_2CO_3.

c. [illegible]at to 200 C in an oven for 30 minutes. Stir and reheat for 30 minutes. Cool in a desiccator. Remove, weight accurately.

d. Dissolve in freshly boiled and cooled distilled water; transfer

to a 500 ml volumetric flask and dilute to the mark with the boiled and cooled distilled water.

e. Calculate the actual normality of this stock solution as follows:

$$N = \frac{\text{gm of } Na_2CO_3}{26.5}$$

For example, if the weight in Section 3 was 27.162 gm Na_2CO_3 the stock solution was:

$$N = \frac{27.162}{26.5} = 1.025N$$

Preparation of 0.0454N Na_2CO_3 from the stock solution:

f. The ml of stock standard solution required to make 1 liter of 0.0454N Na_2CO_3 is calculated as follows:

$$\frac{0.0454N}{\text{Normality of stock solution}} \times 1000$$

For example, if the stock solution was 1.025N Na_2CO_3, then

$\frac{0.0454}{1.025} \times 1000 = 44.3$ ml of 1.025N Na_2CO_3 required to make 1 liter of 0.0454N Na_2CO_3.

g. Measure accurately the required number of ml of the stock solution from a burette into a 1 liter volumetric flask.[1] Dilute to the mark with freshly boiled and cooled distilled water. Stopper tightly with a rubber stopper.

Alternate:

0.0227 N NaOH may be used in place of 0.0454 N Na_2CO_3 where a standard NaOH is available. Titration and calculations are the same with both.

---

1. The indicator may be included in the 0.0454 N Na_2CO_3 by adding 16 ml phenolphthalein indicator before dilution to 1 liter.

2. Standard 0.0167N HCl:

This standard is most accurately prepared from a constant boiling mixture as follows:

Stock Solution of Standard HCl:

a. Place 100 ml pure concentrated HCl and 400 ml distilled water in a beaker.

b. Boil under hood until half the solution has evaporated.

c. Record the barometric pressure during boiling.

d. The remaining solution from (b) will have a constant composition as indicated in the following table:

Barometric pressure	Per cent HCl in solution	Grams solution* required for 1 liter of 1.0 N HCl
770	20.197	180.407
760	20.221	180.193
750	20.245	179.979
740	20.269	179.766
730	20.293	179.555

* Corrected for weighing in air.

Values for intermediate barometric pressures are determined by interpolation.

Weight accurately and rapidly approximately 190 ml of the acid solution and place in a glass-stoppered volumetric flask.

Dilute to 1 liter with recently boiled and cooled distilled water.

Determine the normality of the above stock standard solution as follows:

$$N = \frac{\text{Actual weight HCl solution}}{\text{gm solution required for 1N HCl (from Table, Step d)}}$$

For example, if the barometric pressure during boiling was 740 (Step c), then 179.766 gm of the solution was required to make 1 liter of 1.0 N HCl (Step d). If the actual weight of the acid solution was 195.120 (Step e), then the normality of the stock solution was:

$$\frac{195.120}{179.766} = 1.085N$$

Preparation of 0.0167N HCl from the Standard Stock Solution:

g. The ml of standard stock solution required to make 1 liter of 0.0167N HCl is calculated as follows:

$$\frac{0.0167}{\text{Normality of stock solution}} \times 1000$$

For example, if the stock solution is 1.085 N HCl, then, $\frac{0.0167}{1.085} \times 1000 = 15.4$ ml of 1.085N HCl is required to make 1 liter 0.0167N HCl.

h. Measure accurately from a burette the required number of ml of the standard stock solution into 1-liter volumetric flask with a ground glass stopper. Dilute to the mark with freshly boiled and cooled distilled water.

3. Indicator: Make either a. or b.

a. Methyl Orange - Xylene Cyanole Indicator:

Dissolve 0.2 gm methyl orange plus 0.2 gm xylene cyanole in 100 ml distilled water. Its end point is a grey color at pH 4.5. In

more alkaline waters it is green and in more acid waters, it is red. The end point is easy to detect.

b. Methyl Orange Indicator:

Dissolve approximately 0.2 gm methyl orange in 100 ml water. This indicator has its transition point at pH 4.5, and at this point has an orange color. In a more acid range it is red and in a more alkaline range, it is yellow. The end point is difficult to detect.

4. Phenolphthalein Indicator:

Dissolve approximately 0.05 gm phenolphthalein in 25 ml of 50% alcohol. This indicator gives a pale pink color at pH 8.3, its transition point. In more acid solutions it is colorless and in more alkaline solutions it is red.

Procedures For Free Carbon Dioxide:

Pretest 2 small water samples of approximately 20 ml by adding several drops of methyl orange-xylene cyanole (or methyl orange) to one and several drops phenolphthalein to the other. If the sample is acid to methyl orange-xylene cyanole, acidity is due to acids stronger than CO_2. If the samples are alkaline to methyl orange and acid (colorless) to phenolphthalein, the acidity is assumed to be due to carbon dioxide.

1. In the latter case, carefully pipette 200 ml of the water sample into an Erlenmeyer flask.
2. Add 10 drops phenolphthalein. (The solution should remain colorless.) If the Standard Na_2CO_3 contains the indicator, addition of phenolphthalein to the sample is unnecessary.

3. Titrate to a faint pink (approximately pH 8.3) with 0.0454N sodium carbonate.

4. The number of ml of 0.0454N sodium carbonate x 5 = ppm free CO_2.

5. To differentiate between acidity due to free CO_2 and that due to strong acids (usually mineral acids), proceed as follows:

   a. Pipette duplicate water samples of 200 ml in each of 2 flasks.

   b. Bring one sample to boiling and cool.

   c. Add 10 drops phenolphthalein to each sample.

   d. Titrate each sample to a faint pink (pH 8.3) with 0.0454N Na_2CO_3. Difference in the titres unboiled sample - boiled sample is acidity due to CO_2. Calculate as in 4 above. The titre of the unboiled sample represents total acidity, while that of the boiled sample usually is due largely to mineral acids. Sulfuric acid is the most common mineral acid found in natural waters.

Procedures for Alkalinity, CO_3^{--}, HCO_3^{-}, and OH^{-}

1. Determination of phenolphthalein alkalinity:

   a. To 100 ml of the sample water, add a few drops of phenolphthalein.

   b. If the solution remains colorless, no phenolphthalein alkalinity is present. Continue with Section 2-a.

   c. If a pink color develops upon the addition of phenolphthalein, titrate with 0.0167N HCl, adding a drop every 2 to 3 seconds until the pink color disappears. Record the burette reading as phenolphthalein alkalinity (P).

   d. Save the sample for titration under Section 2-b.

2. Determination of bicarbonate alkalinity:

a. To the water sample from 1-b above, add several drops methyl orange or methyl orange-xylene cyanole and titrate with 0.0167N HCl to the end point (orange color, or grey color respectively). Record the burette reading as methyl orange titre (M).

b. To the water sample from 1-c above, add several drops of methyl orange-xylene cyanole or methyl orange indicator and titrate with 0.0167N HCl to the end point. Record burette reading as methyl orange titre (M).

If the titre is large, it is best to titrate to the end point, then boil to expel CO_2 and retitrate to the end point. The presence of large amounts of CO_2 may give a premature end point if this is not done.

Calculations:

A. If M is larger than P, subtract M-P = X, then:

1. Multiply P ml by 10 to obtain the concentration of CO_3^{--} ions as ppm in the water sample.

2. Multiply X ml by 10.1 to obtain the concentration of HCO_3^- ions as ppm in the same sample.

B. If M=P, then there is no bicarbonate (HCO_3^-) alkalinity, but only CO_3^{--} alkalinity. Multiply P ml by 10 to obtain the concentration of CO_3^{--} as ppm in the water sample.

C. If P is larger than M, subtract P-M = Y then:

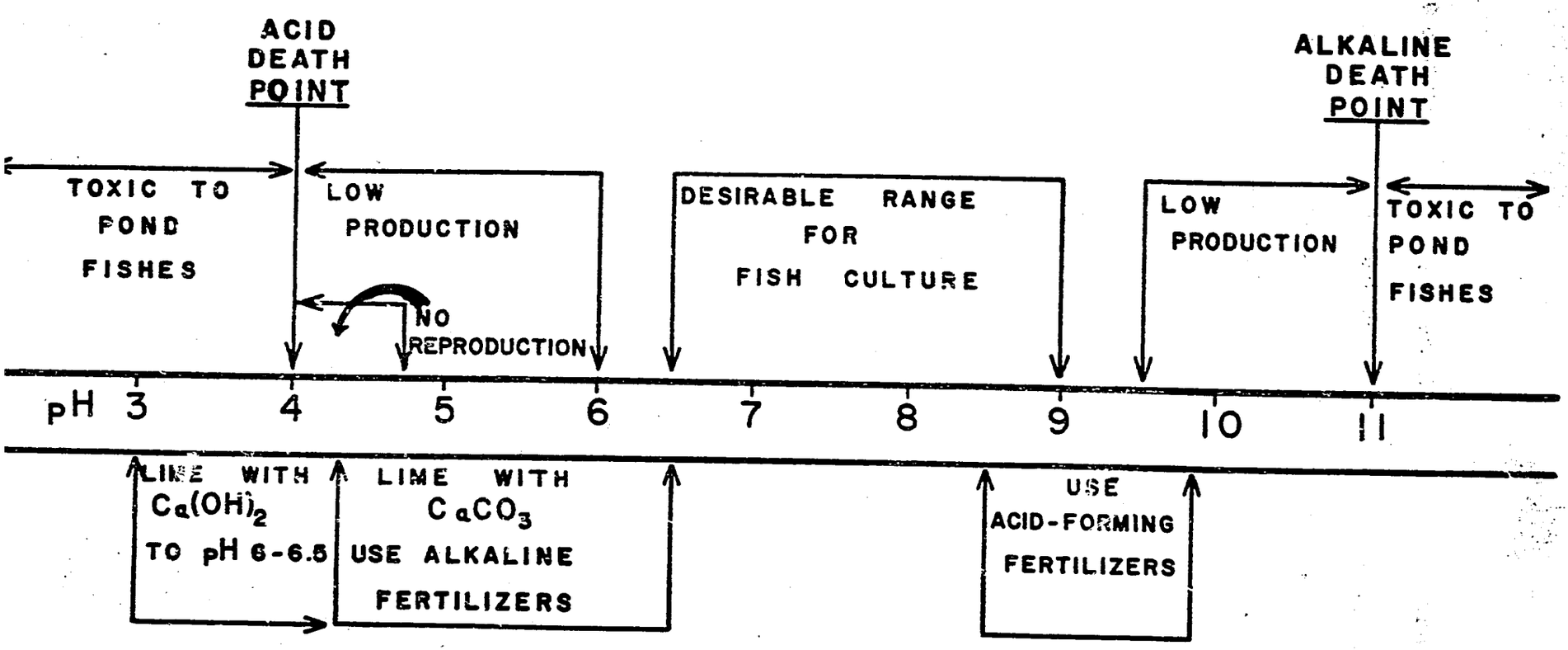
1. Multiply M by 10 to obtain the concentration of CO_3^{--} ions as ppm in the water sample.

2. Multiply Y by 2.84 to obtain OH^- as ppm, or multiply by 5.0 to express OH^- alkalinity as the equivalent in ppm CO_3^{--}.

Total alkalinity expressed as CO_3^{--} equivalent is equal to 5 (M + P).

Total alkalinity expressed as $CaCO_3$ equivalent is equal to 8.35 (M + P).

FIGURE 2. RELATIONSHIP OF pH OF POND WATERS TO THEIR SUITABILITY FOR FISH CULTURE



pH

There is only one satisfactory method of determining pH of pond waters and that is by the use of the electrometric glass electrode. Laboratory and portable models are available.

Universal indicator dyes are available which give different colors over the entire range of pH. The color produced is then compared to a color chart. Errors in determining pH by this method in natural waters vary from 1.0 to 3.0.

Colorimetric determinations using pH indicators, with solutions of known pH for comparison are more accurate, but certain waters may give colors varying 1.0 from the true pH value.

Overlapping indicators, as shown on the following sheet, may be used for approximations of pH, but are subject to errors of the type mentioned above.

Indicator papers are inaccurate, especially in poorly buffered waters. They are useful only to obtain approximate pH values.

For purposes of water classification, the pH of waters should be taken preferably just before daybreak, or before active photosynthesis has materially reduced the available free and/or half-bound carbon dioxide.

FIGURE 3. OVERLAPPING INDICATORS

Their pH at color changes in pond waters

Thymol Blue	
Red	Orange
	Yellow
1.8	2.4

Methyl Orange	
Red	Orange
3.1	4.4

Brom Phenol Blue	
Yellow	Purple
3.4	3.8

Methyl Red	
Red	Yellow
4.8	6.0

Brom Cresol Purple	
Yellow	Purple
5.4	6.4

Brom Thymol Blue		
Yellow	Green	Blue
5.4	6.4	7.0

Phenol Red	
Yellow	Pink
6.8	7.4

Cresol Red	
Yellow	Purple
7.0	8.2

Phenolphthalein	
Colorless	Pink
8.0	9.0

Thymol Blue	
Yellow-green	Blue
8.4	9.0

Thymolphthalein	
Colorless	Blue
9.0	10.5

AMMONIA NITROGEN

Soluble ammonia nitrogen forms a yellow to orange color with Nessler's reagent, the depth of color being proportional to the amount of NH_4^+ present.

Reagents:

1. Standard ammonium chloride solution containing 5 ppm N:

   Since the amount of NH_4Cl required to make a liter of solution containing 5 ppm N (or 0.005 gm N per liter) is small,

   $$\begin{array}{ccc} NH_4Cl & --- & N \\ 53.5 & & 14 \end{array}$$

   $$0.005 \text{ gm} \times \frac{53.5}{14} = .019105 \text{ gm } NH_4Cl,$$

   it is better to prepare first a more concentrated stock solution.

   a. Weigh accurately 1.9105 gm of pure NH_4Cl. Dilute to 1 liter with ammonia-free distilled water.

   b. Measure with a volumetric pipette 10 ml of the above stock solution into a glass-stoppered volumetric flask and dilute to 1 liter with ammonia-free distilled water. This standard solution contains 5 ppm N in the form of NH_4^+.

2. Ammonia-free distilled water: Test distilled water with Nessler's reagent as described below. If no ammonia N can be detected, it may be used directly. If traces of ammonia N are present, acidify with 5 ml concentrated H_2SO_4 per liter. Distill off three-fourths the water into a glass-stoppered bottle and label. The ammonia will remain in the acid water.

3. Nessler's reagent: This reagent may be purchased ready-made or may be made as follows:

   a. Dissolve 100 gm HgI_2 and 70 gm KI in 200 ml distilled water.

   b. Dissolve 160 gm NaOH in 500 ml distilled water, allow to cool.

   c. Add HgI_2 plus KI solution slowly, with stirring, to NaOH solution.

   d. Dilute to 1 liter and store in pyrex out of direct sunlight. It should be stable for up to one year.

Determination:

(For waters free of hydrogen sulfide and other interfering substances.)

1. Filter water sample to remove particulate matter.

2. Pour 50 ml of filtered water sample into 50 ml Nessler tubes.

3. Freshly prepare in 50 ml Nessler tubes 50 ml of standard solutions containing 0.1, 0.25, 0.5, and 1.0 ppm N as NH_4^+ by diluting the 5 ppm standard prepared under 1-b with ammonia-free distilled water as follows:

For standards with following ppm N	Dilute following amounts of standard NH_4Cl solution (containing 5 ppm N) to 50 ml
0.10	1.0 ml
0.25	2.5 "
0.50	5.0 "
0.75	7.5 "
1.0	10.0 "

4. Add to each sample and to each of the above standards 2 ml Nessler's reagent. Invert to mix and let stand 10 minutes for full color to develop.

5. Compare the water sample with the standards. Estimate the ammonia as ppm N.

The comparison should be made by placing a light behind the rack containing the Nessler tubes and comparing only the color observed by looking from the side of the tube directly across the meniscus at the top of the liquid column. This minimizes errors due to precipitates and off-colors in the sample and sometimes will be found to give results approximating those obtained by distillation of the ammonia and subsequent nesslerization.

6. If the sample contains more than 1.0 ppm N, a second sample should be taken, diluted with 4 parts or more of ammonia-free water, and subsequently nesslerized as in Section 4.

Interfering substances

Hydrogen sulfide, sulfides, SO_2, amines, proteins or aldehydes, may cause greenish off-colors. Calcium, magnesium and iron may cause precipitates or turbidities. Interference from these sources can sometimes be overcome by the following pre-treatment of the water. (However, it is preferable to use distillation.)

Reagents:

1. $ZnSO_4$: Dissolve 10 gm $ZnSO_4 \cdot 7\ H_2O$ in ammonia-free water and dilute to 100 ml.
2. NaOH: Dissolve 25 gm NaOH in ammonia-free water and dilute to 100 ml.

3. Rochelle salt solution: Dissolve 50 gm of potassium-sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) in 100 ml and boil until free of ammonia. Dilute to 100 ml.

4. Lead acetate: Dissolve 2 gm in 100 ml distilled water.

Procedure:

1. Remove residual free chlorine with a few drops of 0.025 N sodium thiosulfate (oxygen reagent).
2. Add several drops lead acetate solution and 1 ml $ZnSO_4$ solution to 100 ml of the water sample. Mix.
3. Add 0.5 ml NaOH to a pH of 10.5. Mix. The precipitate should fall, clearing the water and removing proteins, amino acids, sulfides and SO_2.
4. Remove 50 ml of the supernatant liquid to a Nessler tube, add 5 to 6 drops of Rochelle salt solution to remove Ca and Mg.
5. Nesslerize the above solution and compare with standards.

Distillation

Where interfering substances are present, the most accurate method is to remove the ammonia by distillation. Since this is time-consuming, it is not ordinarily done unless very accurate results are needed, or the methods given above do not work.

Reagents:

1. Phosphate buffer (pH 7.4): Dissolve 14.3 gm KH_2PO_4 and 90.1 gm $K_2HPO_4 \cdot 3H_2O$ in distilled water and dilute to 1 liter.
2. Dilute HCl: Dilute 8.5 ml conc. HCl to 1 liter (approximately 0.1 N).
3. 10% NaOH: Dissolve 100 gm NaOH in distilled water and dilute to 1 liter.

Procedure:

Use the Kjeldahl Distillation Setup as shown on the following page.

1. Place 200 ml of the water sample in a distillation flask.
2. Neutralize to litmus paper with a few drops of 10% NaOH. Add a few boiling beads or chips and 5 ml phosphate buffer to give a pH of 7.4. In very hard water (250 ppm Ca or more) more buffer may be needed; add up to 20 ml buffer and adjust pH to 7.4 with acid or base.
3. Distill into collecting flask by bubbling through 10 ml of the dilute HCl until you have collected about 80 ml of the distillate.
4. Dilute to 100 ml, remove 50 ml and nesslerize.
5. Save residue in distillation flask for organic nitrogen determination.
6. Compare with standards (page 28) visually or with spectrophotometer (page 33).
7. Multiply ppm NH_3 measured by nesslerization by 0.5 to get correct ppm in the original sample.

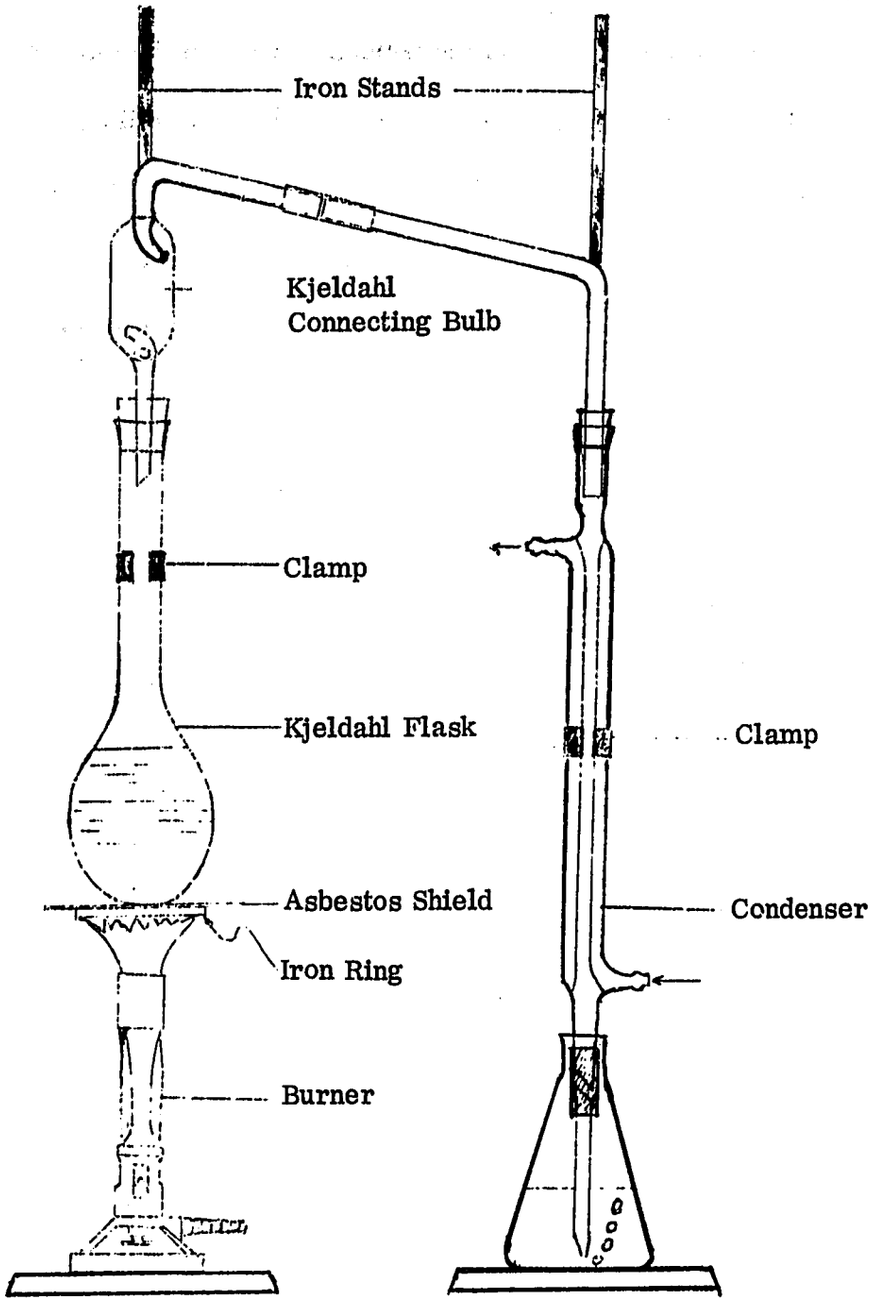


FIGURE 4. KJELDAHL DISTILLATION SETUP

AMMONIA NITROGEN SPECTROPHOTOMETER PROCEDURE

These directions are written for use with a Bausch and Lomb Spectronic 20 colorimeter but can be used with other spectrophotometers with little modification. For best results, it is advisable to read the instruction manual for the instrument to know how to use it properly. The following stepwise procedure is recommended:

1. Turn on machine and warm it up at least 15 minutes.
2. Check to see that photocell 5581 is in place, use a wavelength in the range of 400 to 450 millimicrons, preferably 425. The exact wavelength is not a critical factor, but all determinations must be made at the same wavelength.
3. Set needle at 0% transmittance using amplifier knob on left.
4. Insert blank (cuvette of distilled H_2O) and set needle at 100% transmittance using light control knob on right.
5. Remove blank, needle should return to 0. If it does not, repeat steps 3 and 4 until no further adjustment is necessary. Since the needle may have a tendency to drift, it is advisable to repeat steps 3 and 4 periodically during use.
6. Prepare a series of standards and samples as directed on page 28 , or use samples from distillation (page 31).
7. If there is a significant color in the samples before adding Nesslers reagent, place a portion of the sample in a cuvette and read per cent transmittance. This is the sample blank.

8. Since color development changes with time, the development time between adding Nessler's reagent and reading the color should be constant. Ten minutes is recommended. Add the Nessler's reagent to the first sample and mix. Record the time. After a suitable interval, add Nessler's reagent to the second sample. Repeat with each sample at the same interval. The interval is determined by the speed at which the operator can read the spectrophotometer in step 9 (using a single cuvette an operator can read a sample every 30 seconds with a little practice).

9. Ten minutes after adding Nessler's reagent to the first sample, transfer it to a cuvette and read the per cent transmittance. Repeat with each sample using the interval between samples used in step 8. In this way, the color develops for ten minutes in each sample.

10. Convert per cent transmittance to optical density using the table in the Appendix, page 114

11. Plot optical density of the standards against ppm of the standards on regular graph paper. The calibration curve is the straight line that best fits the points. Record wavelength and development time used on graph for future use of calibration curve. Above about 1.2 to 1.5 ppm, the straight line relationship does not hold. More concentrated samples should be diluted to bring them below this range before nesslerization.

12. Subtract optical density of sample blank from that of sample. Read ppm of samples and reagent blanks directly from calibration curve. Subtract ppm of reagent blank from that of sample after adjusting sample ppm for whatever dilution of the sample was necessary.

ORGANIC NITROGEN

Reagents:

All the reagents listed for the distillation and nesslerization of ammonia (pages 27, 28, and 30) are required, plus the following:

1. Digestion reagent a.: Dissolve 134 gm K_2SO_4 in 650 ml H_2O + 200 ml conc. H_2SO_4. b.: Dissolve 2 gm HgO in 25 ml 6N H_2SO_4. Add "b" to "2" with stirring, dilute to 1 liter. Store above 14 C.
2. Phenolphthalein indicator: Dissolve 0.5 gm phenolphthalein in 100 ml distilled water. Add dilute NaOH until faint pink color appears.
3. NaOH - Na thiosulfate reagent: Dissolve 500 gm NaOH and 25 gm $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter distilled water.

Procedure:

1. Follow procedures for distillation of NH_3 and save residue in Kjeldahl flask.
2. To cooled residue add 50 ml digestion reagent and mix.
3. Heat under hood until white SO_3 fumes are given off and sample clears.
4. Digest for additional 30 minutes and allow to cool.
5. Dilute with 200 ml H_2O, add 0.5 ml phenolphthalein indicator and mix.
6. Add 50 ml hydroxide-thiosulfate reagent, pouring down side of flask.
7. Connect to distillation apparatus and stir to mix the 2 layers. Mixture will be black with a red tint. Add more hydroxide-thiosulfate reagent if red color does not appear.
8. Distill, collect about 130 ml in 20 ml 0.1 N HCl (or H_2SO_4) and dilute to 200 ml.

9. Simultaneously subject 200 ml nitrogen-free water to same procedure to be used as reagent-blank.

10. Nesslerize and evaluate concentration by spectrophotometer as per ammonia determination (pages 43, 44).

NITRITE NITROGEN

Since nitrites may be oxidized readily to nitrates, samples of water should be analyzed promptly. The sulfanilic acid method is used.

Reagents:

1. Sulfanilic acid solution*: Dilute 30 ml glacial acetic acid to 100 ml with distilled water. Add 1 gm sulfanilic acid.
2. Alpha-naphthylamine solution*: Dilute 30 ml glacial acetic acid to 100 ml with distilled water. Add 0.5 gm alpha-naphthylamine. This reagent is not stable.
3. Stock $NaNO_2$ solution: Dissolve 1.230 gm $NaNO_2$ in 500 ml distilled water.
4. Standard $NaNO_2$ solution: Using volumetric pipette dilute 50 ml of the stock $NaNO_2$ solution to 500 ml. Then take exactly 5 ml of this dilute solution and dilute again to 500 ml. This solution contains 0.5 ppm N as NO_2^-.

Procedure:

1. Filter 50 ml of the water sample into a Nessler tube.
2. Add 1 ml sulfanilic acid solution.
3. Add 1 ml alpha-naphthylamine solution and mix. This gives a pink to red color within 10 minutes if NO_2^- is present.
4. Compare with standard diluted from the standard $NaNO_2$ solution and similarly treated.

---

* A dry reagent may be prepared by grinding 1 gm alpha-naphthylamine, 10 gm sulfanilic acid and 89 gm tartaric acid in a mortar. Use 0.3 gm in 50 ml water sample. This reagent is stable.

Number of ml standard $NaNO_2$	ppm N when diluted to 50 ml
1	0.01
2	0.02
3	0.03
4	0.04
5	0.05

If measured in a spectrophotometer, set the wavelength at 530.

NITRATE NITROGEN

The phenoldisulfonic acid method is used. Nitrates react to form the tripotassium salt of nitrophenoldisulfonic acid.

If the chloride content is greater than 30 ppm, the chlorides should first be precipitated by adding several drops of silver sulfate (5%) and filtering.

Nitrites, if in excess of 0.2 ppm should first be determined and converted to nitrates by heating with 5 ml H_2O_2 to each 100 ml of water sample. The amount of nitrate (NO_3^-) formed by oxidation of NO_2^- should be subtracted from the final results.

Reagents:

1. Phenoldisulfonic acid solution: This acid may be purchased or prepared as follows: Dissolve 25 gm of pure white phenol in 150 ml of concentrated sulfuric acid, add 75 ml of fuming sulfuric acid (13-15 per cent SO_3), and heat at 100 C for 2 hours. Store in a brown bottle.

2. Standard nitrate solution: Dissolve 0.607 gm of pure sodium nitrate in 1 liter of nitrate-free distilled water. Evaporate 50 ml of this solution to dryness in a porcelain dish; when cool, treat with 2 ml of the phenoldisulfonic acid solution, rubbing with a glass rod to insure intimate contact, and dilute to 500 ml with distilled water. One ml is equivalent to 0.01 mg of nitrogen as nitrate. (This solution is permanent.) Prepare standards for comparison by adding strong ammonium hydroxide (1-1) to measured volumes of the standard solution in 100 ml Nessler tubes. The intensity of the yellow color increases with increasing NO_2^- concentration.

3. Ammonium hydroxide: Dilute 500 ml NH_4OH to 1 liter.

4. Prepare standards as follows:

Standard nitrate	Diluted to	Equal to ppm
0.1 ml	100 ml	0.01
0.5 ml	100 ml	0.05
1.0 ml	100 ml	0.10
2.0 ml	100 ml	0.2
5.0 ml	100 ml	0.5
10.0 ml	100 ml	1.0

Determination:

1. Filter 100 ml water sample and place in porcelain evaporating dish.
2. Place on steam bath and evaporate to dryness.
3. Cool, add 2 ml phenoldisulfonic acid and mix with a glass rod.
4. Dilute with approximately 10 ml water.
5. Add NH_4OH (1-1) until maximum color develops. Transfer to 100 ml Nessler tube and dilute with distilled water to the mark.
6. Compare with standards prepared in Section 4 above visually or with spectrophotometer (page 51).

NITRATE NITROGEN DETERMINATION BY USE OF SPECTROPHOTOMETER

1. Prepare standards of known concentrations as described in Reagents section 4 (page 50). Concentrations of 0.01, 0.05, 0.10, 0.15 and 0.20 ppm are suggested.

2. Switch on spectrophotometer to warm up machine for at least 15 minutes. Using a wavelength within a range of 400 to 450 millimicrons, with 5581 photocell, set machine to zero optical density by using distilled water. Subsequently, take optical density readings for the known standards. From the values obtained compute conversion factor by dividing each concentration by corresponding optical density. The average of these quotients is the conversion factor (F). Also, plot optical density and concentration on regular graph paper.

3. To analyze unknown sample, follow steps 1-5 of Determination on page 50.

4. Transfer treated sample obtained in step 5 to cuvette and take optical density reading after setting machine to zero optical density with distilled water. For blank, use sample untreated with NH_4OH.

5. Correct the sample reading by subtracting the blank reading and read the concentration directly from the graph or multiply the corrected optical density reading by the conversion factor (F) to give the concentration of nitrates in ppm of the sample.

TOTAL NITROGEN

Total nitrogen concentration in the water includes that in both inorganic and organic compounds, but excludes elemental N gas.

Reagents:

1. Concentrated sulfuric acid-salicylic acid solution: Measure 900 ml of conc. sulfuric acid into a large clean beaker. Add 72 gm salicylic acid, stir the salicylic acid powder carefully into the acid until completely dissolved.
2. Sodium thiosulfate - crystalline, C.P.
3. Sodium sulfate-mercuric oxide powder: Mix 454 gm sodium sulfate and 24.4 gm mercuric oxide.
4. Granulated zinc
5. Sodium hydroxide-sodium sulfide solution: First, prepare approximately 16 N sodium hydroxide by dissolving 640 gm NaOH in water and diluting to 1 liter.

   Prepare sodium sulfide solution by dissolving 34 gm Na_2S in 85 ml of distilled water. Dilute to 1,000 ml with 16N NaOH.
6. Nessler reagent: As in ammonia analysis.

Procedure:

The method described here is essentially the Kjeldahl method for nitrogen analysis especially adapted for pondwater analysis.

1. Shake water sample to bring all particulate matter into uniform suspension. Measure carefully 100 ml of water sample into a clean 500 ml Kjeldahl

flask. Add 16 ml of concentrated sulfuric-salicylic acid solution and shake well. Allow to stand for at least 30 minutes.

2. Add 5 gm (1 teaspoon) of sodium thiosulfate crystals and shake well. Heat flask under hood until white fumes begin to evolve profusely. This takes about 45 minutes to one hour. Use asbestos shield under flask.
3. Add 5 gm (1 teaspoon) sodium sulfate-mercuric oxide powder. Continue heating sample until the liquid is clear. Continue digestion for another 30 minutes. Cool.
4. Add 250 ml ammonia-free distilled water (page 37). Add 1/2 teaspoon granulated zinc. Alkalize with 60 ml NaOH-Na_2S solution. Do this by adding the alkali carefully along the side of flask to avoid violent reaction.
5. Set up Kjeldahl distillation apparatus, consisting of condenser, condenser bulb, receiving flask, and the Kjeldahl flask with the sample (page 42). Check that the fittings are airtight. Shake the sample to mix thoroughly when fitting is made.
6. Collect 225 ml of distillate in 20 ml 0.1 N HCl. This takes about 1 to 2 hours. Dilute to 300 ml.
7. Simultaneously with the sample analysis, subject 100 ml of nitrogen-free distilled water to the same treatment procedure as above to be used as reagent blank.
8. The total nitrogen in the original 100 ml sample is now all converted into ammonia in the 300 ml distillate. Nesslerize a 50 ml portion and evaluate concentration by using the spectrophotometer (Ammonia analysis, page 43).
9. Multiply ppm N measured by nesslerization by 3 to obtain ppm in sample.

PHOSPHATES

This method is for the determination of phosphates in solution. It cannot be used in waters containing more than 0.2 ppm arsenates or arsenites as these ions give a blue color similar to the phosphate ion.

Reagents:

1. Ammonium molybdate-sulfuric acid solution: Dissolve 25 gm ammonium molybdate in 200 ml of water heated to 60 C and filter. Pour 280 ml arsenic- and phosphorus-free concentrated sulfuric acid slowly and carefully into 420 ml distilled water. After both solutions have cooled, add the ammonium molybdate solution, slowly with shaking, to the sulfuric acid solution. Cool, dilute to 1 liter with distilled water. This is approximately a 10 N sulfuric acid solution containing approximately 2.5 gm of ammonium molybdate per 100 ml.

2. Stannous chloride solution: Dissolve 1 gm $SnCl_2 \cdot 2H_2O$ in 5 ml of concentrated HCl. Dilute to 50 ml with distilled water. Filter into a 125 ml aspirator bottle and cover the liquid surface with white mineral oil. The aspirator bottle tubulature may be fitted with a rubber tube, drawn out glass tubing and pinchcock for drop-by-drop delivery of the solution. This solution deteriorates. When a blue color fails to develop after its addition to the molybdate-treated phosphate solution, discard and make up fresh.

3. Standard phosphate solution: Dissolve 0.2195 gm of pure potassium-dihydrogen-phosphate (KH_2PO_4) and dilute to 1,000 ml with distilled water.

This solution contains 50 ppm phosphorus and is too concentrated to use directly. A second stock solution is made by taking 50 ml of the first stock solution and diluting to 500 ml. This second stock solution contains 5 ppm P and is used for making the standard solutions for comparisons.

Procedure:

1. Using the standard phosphate (containing 5 ppm P), prepare standards containing 0.025, 0.05, 0.10, 0.25, 0.5, 0.75, and 1 ppm P as follows:

For standards containing the following ppm P	Dilute following number of ml of the standard phosphate (containing 5 ppm P) to 50 ml
0.025	0.25
0.05	0.5
0.10	1.0
0.25	2.5
0.50	5.0
0.75	7.5
1.00	10.0

2. Add to each standard 2 ml of the ammonium molybdate-sulfuric acid solution and mix by shaking.

3. Add 5 drops $SnCl_2$ solution and shake. A blue color should develop promptly with the intensity of color increasing with increase in phosphorus.

   If a blue color does not develop, the stannous chloride solution has deteriorated and a fresh one must be prepared.

   The blue color begins to fade in 10 to 12 minutes and an additional drop of stannous chloride should be added to bring the full color back.

4. Measure 50 ml of the water to be tested and place in a 100 ml Erlenmeyer flask.

5. Add 2 ml of the ammonium molybdate-sulfuric acid and mix by shaking.

6. Add 5 drops of $SnCl_2$ solution and shake.

7. Compare the color produced by the phosphorus in the sample with that in the standards and estimate the ppm P.

   The comparison can be made in 50 ml Nessler tubes or directly in the Erlenmeyer flasks, or by use of the spectrophotometer as described on the following pages.

PROCEDURE FOR MAKING THE CALIBRATION CURVE IN THE SPECTROPHOTOMETRIC METHOD OF PHOSPHORUS ANALYSIS

1. Switch on the "Spectronic 20 Colorimeter" for warm-up period of about 15 minutes before using. Be sure to use the 1P40 photocell and a red filter. Set the wavelength at 690 millimicrons. (Read the Reference Manual carefully before using the instrument.)
2. Using the standard phosphate, prepare standards containing 0.025, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 ppm P as described in Section 1 on page 55 .
3. Add 2 ml ammonium molybdate-sulfuric acid to each standard.
4. Have 8 clean, dry cuvettes ready.
5. Place approximately 40 ml of the standards blank into one of the cuvettes. (The blank here is 50 ml of distilled water as was used in preparing the standards, plus 2 ml ammonium molybdate plus 5 drops stannous chloride.
6. Insert the cuvette into the sample holder of the spectrophotometer, being careful to set the index line on the cuvette opposite the index line on the sample holder.
7. Adjust the instrument to 100% transmittance (or 0 optical density) with the blank.
8. Add 5 drops stannous chloride to each of the standards treated with molybdate in Section 3.
9. A blue color should develop promptly which intensifies for a period and then slowly becomes less intense. Set up so that readings on the spectrophotometer are made just 10 minutes after the stannous chloride

is added. (If no color develops, make up fresh stannous chloride.)

10. Place approximately 40 ml of each standard in cuvettes and read per cent transmittance and optical density. Measure per cent transmittance and optical density of each standard successively and as quickly as possible.

11. Plot the optical density (ordinate) against the corresponding concentration (abscissa) in standard graph paper. The curve. a straight line passing through the origin, is the calibration curve.

12. To 50 ml of the test water, add 2 ml ammonium molybdate-sulfuric acid. Place 40 ml in a cuvette and reset the spectrophotometer at 100% transmittance as in Section 7 above: This is the blank for the test water.

13. To each 50 ml sample, add 2 ml ammonium molybdate and 5 drops stannous chloride.

14. Place in a cuvette, read 10 minutes after the stannous chloride was added and compare to the curve for the standards (Section 11).

TOTAL HARDNESS

Hardness of water is due principally to soluble salts of divalent cations, principally calcium and magnesium. Temporary hardness is due to calcium and magnesium bicarbonates. This is called "temporary" because upon boiling, the bicarbonates change to carbonates and part of the calcium and magnesium are precipitated.*

$$Ca(HCO_3)_2 \rightarrow CaCO_3 + H_2O + CO_2$$

Permanent hardness is due principally to soluble Ca and Mg carbonates and salts of inorganic acids (e.g. $CaSO_4$)*. Permanent plus temporary hardness = total hardness.

Principle: The indicator forms a wine-red color with Mg ions. A standard solution of the sodium salt of ethylenediamine tetraacetic acid (sometimes called versene) is used for titration. It combines first with free Ca ions, next with free Mg ions, and, finally at the end point extracts Mg from the indicator thus changing the color from wine-red to blue.

The blue end-point occurs at pH 7.0 - 10 and a buffer solution must be used which keeps the pH at approximately 9 - 10. This is necessary because the indicator has two color changes:

wine-red $\leftrightarrow$ blue $\leftrightarrow$ orange
pH 6.3 pH 11.5

*Solubility at 25 C of $CaCO_3$ is 14 ppm; of Mg CO_3 - 106 ppm; of $CaSO_4$ - 2090 ppm.

Reagents:

These reagents can be purchased ready to use or made as follows:

1. Buffer: Mix 67.5 gm NH_4Cl with 570 ml concentrated NH_4OH. Dilute to 1 liter.

2. Indicator: Dissolve 4.5 gm hydroxylamine HCl and 0.5 gm of either Calmagite or Eriochrome black T (also known as Chrome black T or F241) in 100 ml 70% alcohol. Calmagite is more stable and is said to give a sharper end point. Eriochrome black T indicator should be replaced every 2 to 4 weeks.

3. Standard $CaCl_2$: Dissolve 1.00 gm pure $CaCO_3$ in about 10 ml diluted HCl (1-10) and dilute with distilled water to exactly 1 liter. Store in a glass-stoppered bottle. 1 ml = 1.0 mg $CaCO_3$.

4. Standard sodium ethylenediamine tetraacetate (EDTA): Dissolve 4.0 gm disodium dihydrogen ethylenediamine tetraacetate (also known as disodium dihydrogen versenate) and 0.1 gm $MgCl_2 \cdot 6H_2O$ in 750 ml distilled water and dilute to 1 liter in a pyrex volumetric flask. Standardize by titrating against 25 ml of standard $CaCl_2$ solution plus 1 ml of buffer plus 4 drops indicator. Dilute so that 1 ml of standard = 1.0 mg $CaCO_3$.

Procedure:

1. Pipette 50 ml water sample into a 250 ml Erlenmeyer flask. (See step 6.)

2. Add 1 ml buffer and mix.

3. Add 4 drops indicator.

4. Titrate with above standard solution. At the end point, the solution should clear and should change from wine-red to pure blue.

5. Multiply ml standard used x 20 = total hardness as ppm $CaCO_3$.

6. For very soft waters, 200 ml sample with 4 ml buffer may be titrated and the ml of standard required multiplied by 5 to obtain ppm $CaCO_3$.

CALCIUM IN POND WATERS

Reagents:

1. NaOH Solution: Weigh approximately 40 gm pure NaOH and dilute to 1 liter with freshly boiled and cooled distilled water (approximately 1N).

2. Calcium indicator: Mix 0.2 gm Murexide (also called NH_4 purpurate) with 100 gm NaCl. Grind to a uniform mix with a mortar and pestle. (Eriochrome blue black R may be substituted for Murexide; this gives a color change from red to blue whereas the Murexide end-point is orchid-purple and more difficult to detect.)

3. Standard sodium ethylenediamine tetraacetate: (See Total Hardness)

Procedure:

1. Measure 50 ml water into a 250 ml Erlenmeyer flask.

2. Add 2 ml of NaOH solution and stir.

3. Add 0.2 gm calcium indicator and stir. (If Ca is present, color is salmon pink with Murexide).

4. Titrate with standard sodium ethylenediamine tetraacetate, stirring constantly. Towards the end point, the color has a purple tinge. At the end point, the color is orchid-purple.

5. Multiply ml standard required x 20 = Ca (as ppm $CaCO_3$).
   Strontium and barium are included as calcium by this method, but are usually present in very small amounts.

MAGNESIUM IN POND WATERS

Total hardness (as ppm $CaCO_3$) - Ca (as ppm $CaCO_3$) = Mg (as ppm $CaCO_3$).

Mg (as ppm $CaCO_3$) x 0.84253 = Mg (as ppm $MgCO_3$).

This is an estimate only as "hardness" may include other salts in addition to calcium and magnesium. A.P.H.A. lists cadmium, lead and zinc as included in hardness by E.D.T.A. titration.

SALINITY

The average percentage compositions of dissolved solids in river waters and in sea waters are as follows:

Ion	Average river water	Sea water
CO_3^{--}	35.15 (HCO_3^-)	0.41
SO_4^{--}	12.14	7.68
Cl^-	5.68	55.04
NO_3^-	0.90	----
Ca	20.39	1.15
Mg	3.41	3.69
Na	5.79	30.62
K	2.12	1.10
$(Fe,Al)_2O_3$	2.75	----
SiO_2	11.65	----
Sr, H_3BO_3, Br^-	-----	0.31
Total	100.0	100.0

The percentage composition of river waters varies widely, depending upon the soils, the kinds and amounts of pollution and the amount of rainfall on the watershed. Consequently, the total dissolved salt content of fresh waters is best determined by evaporation and incineration of a sample followed by weighing the incinerated salts. Incineration is necessary for the destruction of organic matter. It also converts carbonates and bicarbonates to oxides.

Salinity of sea water is defined as the total amount of salts in

solution expressed as gm per kg of sea water, with all carbonates converted to oxides, all bromides and iodides expressed as chlorides and all organic matter completely oxidized.

In the oceans the percentage composition of dissolved solids is relatively constant. Consequently, it was found possible to relate the chloride content to the total dissolved salt content, or salinity, of sea water.

The relationship is: Salinity = 0.03 plus 1.805 x chlorinity. Chlorinity is the total amount of chloride present in 1 kg of sea water, plus all bromides and iodides expressed as chlorides. Chlorinity is thus determined by titration of sea water with standard $AgNO_3$, using a few drops of K_2CrO_4 as indicator.

The salinity of brackishwaters, where the sea water and river waters are mixing, is also commonly determined by the silver nitrate titration. This is not exactly accurate because in the zone of mixing, the percentage composition of salts in solution is not constant and often differs materially from that in sea water, particularly in areas of low salinity. However, for practical purposes, it appears sufficiently accurate to validate its use biologically.

The reaction is: $NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$

As long as chlorides remain in solution the silver is used to form insoluble AgCl. As soon as the last soluble chloride is precipitated, the following reaction occurs with the potassium chromate.

$$K_2CrO_4 + 2AgNO_3 \rightarrow Ag_2CrO_4 + 2\,KNO_3$$

The Ag_2CrO_4 precipitate has a red color.

Reagents:

1. 0.1595 N silver nitrate: Weigh 27.096 gm C. P. $AgNO_3$ crystals and dilute with distilled water to 1 liter in a volumetric flask. Keep in a dark place.

2. 5% potassium chromate indicator:* Dissolve 5 gm of K_2CrO_4 in 100 ml distilled water.

Procedure:

1. Measure 10 ml of the water to be analyzed into a 125 ml Erlenmeyer flask.

2. Add 5 drops potassium chromate indicator. Use a magnetic stirrer.

3. Titrate with standard 0.1595 N silver nitrate to a permanent pink.

4. Express salinity as parts per thousand ($^{o}/oo$). Each 1 ml of 0.1595 N silver nitrate = 1.0 $^{o}/oo$ or 1 ppt salinity

   For more accurate expression of salinity, the above figure for salinity should be corrected by the following amounts:

Salinity found	Correction to be applied
8	+0.15
10	+0.16
12	+0.19
14	+0.20
16	+0.23
20	+0.23
24	+0.20

*Sodium fluorescinate (also called uranin) is reported to give a more accurate end-point than potassium chromate. The precipitate changes from greenish yellow to pink at the end point.

CHLORIDES

The determination of salinity is essentially the determination of chlorides plus bromides and iodides by precipitation with silver nitrate. Since bromides and iodides are present only as traces in most fresh waters, the silver titration method may be used to determine the chlorides.*

Procedure:

1. Measure 500 ml of the water to be analyzed into a 1000 ml Erlenmeyer flask.
2. Add 5 drops potassium chromate indicator. Use a magnetic stirrer.
3. Titrate with standard 0.1595N silver nitrate to a permanent pink.
4. Each 1 ml of 0.1595N silver nitrate = 5.65 mg chloride ion (Cl^-), or = 9.3 mg chlorides as NaCl.
5. To express as Chloride (Cl^-) in sample:

   ml 0.1595N silver nitrate titre x 5.65 x 2 = ppm Cl^-

   as NaCl equivalent in sample:

   ml 0.1595N silver nitrate titre x 9.3 x 2 = ppm as NaCl.

*In polluted waters containing soluble proteins, this method will give inaccurate results because silver will combine with proteins.

CHLORINE

O-Tolidine Method for Free and Combined Available Chlorine

City water supplies are usually chlorinated for the purpose of disinfection and contain from traces to 2 ppm residual chlorine. Ponds are occasionally chlorinated to control diseases and parasites. Part of the chlorine combines with organic matter and part slowly reacts with water, especially when exposed to sunlight.

$$2\ Cl + H_2O \text{ -------- } HOCl + HCl$$

$$4\ HOCl \text{ -------- } 4\ HCl + 4\ O$$

or

$$4\ HOCl + 4\ HCl \text{ ------ } 4\ H_2O + 8\ Cl$$

$$8\ Cl + 4\ H_2O \text{ ------ } 8\ HCl + 4\ O$$

Free available chlorine is in the form Cl, OCl^- or HOCl; combined available chlorine is present in such forms as the chloramines, which are often used for city water disinfection by reaction of NH_4OH and HOCl, or they may be formed in natural waters:

$$NH_4OH + HOCl \text{ -------- } NH_2Cl + 2H_2O$$ (Only monochloramine formed above pH = 8.5)

$$NH_4OH + 2\ HOCl \text{ ------- } NHCl_2 + 3H_2O$$ (Only dichloramine found when pH = 4.4 - 5.0)

At pH 5.0 to 8.0, mono- and dichloramine occur together.

Residual chlorine is that in free available forms and in combined available forms. The orthotolidine method measures total available residual chlorine. This reagent reacts to give a yellow color, with the intensity

dependent upon the amount of chlorine. The pH during the reaction should be 1.3 or less.

Waters containing more than 0.3 ppm, Fe, 0.01 ppm, Mn (ic) or over 0.1 ppm N (as NO_2^-) give interfering colors. Many pond waters low in oxygen contain sufficient iron to give a yellow color with O-tolidine in the absence of available chlorine.

Reagents:

1. Orthotolidine reagent: Dissolve 1.4 gm orthotolidine dihydrochloride in 500 ml distilled water. Add with stirring to 500 ml of dilute HCl (1-3). Store in glass-stoppered amber bottles in the dark. This solution should be prepared fresh every 5 to 6 months.

2. Permanent color standards for chlorine estimation:

   a. Stock phosphate buffer: Dissolve 11.43 gm anhydrous disodium phosphate (Na_2HPO_4) plus 23.08 gm anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 1 liter water. Allow to stand 48 hours before using.

   b. Phosphate buffer 0.1 M: Filter the above stock phosphate buffer and dilute 200 ml to 1 liter with distilled water. This gives a pH of 6.4.

   c. K chromate - dichromate standard: Dissolve 0.155 gm $K_2Cr_2O_7$ and 0.465 gm K_2CrO_4 with 0.1 M phosphate buffer and dilute to 1 liter with this same buffer. The color is equivalent to 1 ppm available chlorine by the O-tolidine method. Lower standards can be obtained by dilution as follows:

Equivalent to ppm Cl	Ml standard Cr_2O_7 - CrO_4	Diluted with 0.1 M buffer ml
1.0	10	0
0.5	5	5
0.3	3	7
0.2	2	8
0.1	1	9

d. Arsenite reagent: Dissolve 5 gm sodium meta arsenite ($NaAsO_2$) in 1 liter distilled water.

Procedure:

1. Collect in a glass vial 10 ml sample of water to be tested.
2. Add 0.5 ml O-tolidine reagent. Compare the color with permanent standards.

   For field work, or a quick check, the development of even a light yellow color is indicative of sufficient chlorine to be injurious or toxic to fishes.

Alternate Method Where Fe, Mn, or NO_2 Interfere:

1. Add 10 ml of the water sample to 0.5 ml O-tolidine reagent. Mix quickly and in exactly 5 minutes compare with color standards. This represents total residual chlorine plus interfering colors (T).
2. To 0.5 ml arsenite reagent add 10 ml of the water sample. Mix quickly, and rapidly add 0.5 ml O-tolidine. Mix and compare with color standards in exactly 5 minutes. This represents the interfering colors (I).
3. The difference, T-I = total residual chlorine.

Titration Method for Free Chlorine

This method is based on the reaction: $Cl_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaCl$

The indicator used is O-tolidine.

Reagents:

1. O-tolidine reagent as prepared on page 69 .
2. 0.025N sodium thiosulfate as prepared for oxygen analysis on page 8 .

Procedure:

1. To 1,000 ml water sample, add 2 ml O-tolidine reagent to develop a yellow color.
2. Titrate with 0.025N sodium thiosulfate to disappearance of the yellow color.
3. Multiply ml of titre by 0.88 to obtain ppm available chlorine.

Calculation:

1 ml 0.025N sodium thiosulfate = 1 ml 0.025N Cl

1N Cl = 35.46 gm/liter

0.025N Cl = 35.46 x 0.025 = 0.8865 gm/liter

1 ml 0.025N Cl = 0.8865 mg Cl

ARSENIC

Arsenicals are used in ponds for weed control at rates ranging from 2 to 8 ppm As_2O_3. The chemical most commonly used is sodium arsenite, approximately $NaAsO_2$, in an aqueous solution containing either 4 or 8 pounds As_2O_3 per gallon.

After application to the water, the arsenic is rapidly absorbed by the aquatic plants, including plankton, and by the bottom soils. Applications of As_2O_3 in excess of 8 ppm reduced fish production in ponds.

The method here employed is the Gutzeit test because it is specific for arsenic. The colorimetric method given for phosphorus (molybdate-stannous chloride) will also yield a blue color with arsenic and it is occasionally necessary to determine arsenic in order to be sure that it is not being falsely reported as phosphorus.

Arsenic present in aqueous solution can be determined directly. However, that in organic matter must first have the organic matter destroyed. This is done by placing the weighed sample in a Kjeldahl flask, adding 2 to 8 ml concentrated H_2SO_4 and 20 ml concentrated nitric acid. This is boiled to about 15 ml; add perchloric acid and nitric acid drop by drop until the solution clears. The digested sample is washed into a volumetric flask, diluted to a known volume and aliquots taken for arsenic analysis.

Gutzeit Analysis for Arsenic

Reagents:

1. Dilute HCl: Dilute 1 part special arsenic-free HCl with 6 parts distilled water.

2. Zinc: Use 20 mesh metalic zinc, arsenic free.

3. Lead acetate solution: Dissolve 10 gm Pb $(CH_3COO)_2 \cdot 3H_2O$ in 100 ml distilled water.

4. Lead acetate cotton: Soak 2-inch lengths of either absorbent cotton or dentist's roll cotton in the lead acetate solution. Wring out excess moisture and store moist cotton in a wide-mouth bottle.

5. Mercuric bromide solution: Dissolve 5 gm $HgBr_2$ in 100 ml 95% alcohol.

6. Mercuric bromide paper: Purchase commercially prepared Gutzeit arsenic papers (or cut strips to fit the Gutzeit reaction tube from filter paper). Soak in the mercuric bromide solution and dry by draining off excess fluid and waving papers in the air. Store strips after drying in a desiccator placed in the dark.

7. As_2O_3 standard: Dissolve 5.00 gm arsenious oxide (As_2O_3) in 25 ml 10% HCl and dilute to 1 liter. 1 ml = 5 mg As_2O_3.

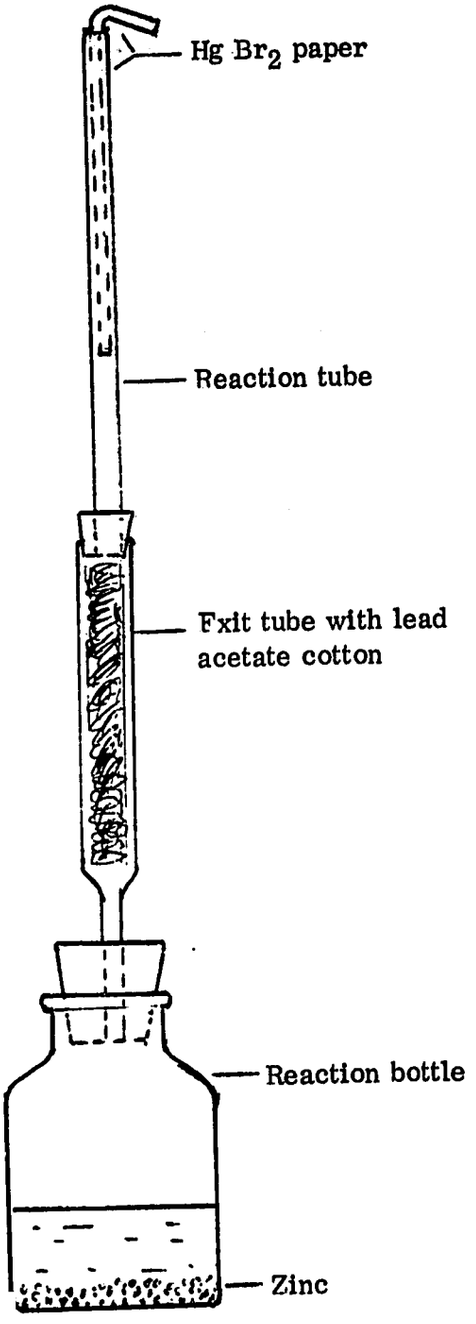


FIGURE 5. GUTZEIT ARSENIC BOTTLE

Procedure:

1. Place lead acetate cotton in the large exit tube as illustrated.
2. Insert a strip of mercuric bromide paper in the reaction tube as illustrated.
3. Place 3 gm metallic zinc in the reaction bottle.
4. Add 15 ml dilute HCl (1-6) to the reaction bottle.
5. Add the aliquot sample and insert the exit tube assembly as illustrated. Be sure to position the reaction tube in a vertical position so that the bromide paper will be colored evenly on both sides.
6. Allow the reaction, which releases arsine, AsH_3, along with the nascent hydrogen, to proceed at room temperature. The arsine reacts with mercuric bromide paper to give an orange to yellow stain on the paper.
7. After 1 to 2 hours, when effervescence has ceased, remove the $HgBr_2$ strips. Measure the stain to the nearest mm on both sides of the paper and compare the average with a graph prepared from known solutions.
8. The standard graph is prepared from the standard solution at 5 mg intervals from zero to 30 mg As_2O_3.

SODIUM, POTASSIUM AND CALCIUM BY FLAME PHOTOMETER

Sodium, potassium and calcium each produce a color (yellow, red-violet and yellow-red respectively) when burned in a flame. In the flame photometer, the solution containing the sample is atomized into an oxygen-gas flame. A filter for the particular element is inserted to allow only passage of the desired wavelength of light and the intensity of color measured by a photometer. The intensity is related to the concentration of the element present in the solution.

Since flame photometers vary greatly in exact method of operation, be sure to read the operating directions for the particular model you are to use.

Reagents and Standards:

1. 1% Sterox stock: Weigh 5 gm Sterox into a 200 ml beaker, add 200 ml distilled water and stir until dissolved. Transfer to 500 ml pyrex volumetric flask and dilute to the mark with distilled water.
2. 0.02% Sterox atomizing solution: Dilute 20 ml of 1% Sterox stock in a 1,000 ml pyrex volumetric flask to 1,000 ml with distilled water.
3. 500 ppm Na standard: Dry reagent grade NaCl by heating for 30 minutes at 125 C. Cool in a desiccator. Place 1.271 gm in a 1,000 ml volumetric pyrex flask, add 900 ml distilled water, shake until dissolved and fill to mark.

Prepare standards as follows:

To obtain ppm Na	Add 1 ml of 1% Sterox plus following ml of 500 ppm Na and dilute to 50 ml
1	0.1
5	0.5
10	1.0
25	2.5
50	5.0
100	10.0

4. 500 ppm K standard: Dry reagent grade KCl for 30 minutes at 125 C and cool in a desiccator. Weight 0.9535 gm and place in a 1,000 ml pyrex volumetric flask. Dissolve and fill to the mark with distilled water.

Make standards by dilution with distilled water as follows:

To obtain ppm K	Add 1 ml of 1% Sterox plus following ml of 500 ppm K and dilute to 50 ml
1	0.1
5	0.5
10	1.0
25	2.5
50	5.0
100	10.0

5. 500 ppm $CaCO_3$ standard*: Dry reagent grade $CaCO_3$ at 150 C for 30 minutes and cool in a desiccator over soda-lime.[1] Weigh 0.500 gm and place in a 1,000 ml pyrex volumetric flask. Add 25 ml dilute HCl (1:1) and swirl until calcium is dissolved. Dilute to 1,000 ml with distilled water.

Make standards as follows:

To obtain ppm $CaCO_3$	Equivalent to ppm Ca	Add 1 ml of 1% Sterox plus following ml 500 $CaCO_3$* and dilute to 50 ml
1	0.4	0.1
5	2.0	0.5
10	4.0	1.0
25	10.0	2.5
50	20.0	5.0
100	40.0	10.0

*The 1,000 ppm $CaCO_3$ standard prepared for the determination of hardness may be used here and would require the dilution of the ml given in the right column of the above table to 100 ml instead of 50 ml to obtain the ppm given in the left column.

6. Standard Na-K-Ca check solution: After the calibration curves are obtained, a 50 ppm and a 100 ppm Na-K-$CaCO_3$ check solution can be used to set the galvanometer scale. These are prepared as follows:

1. A mixture of NaOH-CaO with 18% water. Commercially available as white granules that absorb CO_2.

1. 25 ppm Na-K-$CaCO_3$: Pipette accurately into a 250 ml pyrex volumetric flask,

12.5 ml standard 500 ppm Na

12.5 ml standard 500 ppm K

12.5 ml standard 500 ppm $CaCO_3$

50 ml 1% Sterox

Dilute to 250 ml with distilled water.

2. 100 ppm Na-K-$CaCO_3$: Pipette accurately into a 250 ml pyrex volumetric flask,

50 ml 1% Sterox

50 ml standard 500 ppm Na

50 ml standard 500 ppm K

50 ml standard 500 ppm $CaCO_3$

Dilute to 250 ml with distilled water.

Preliminary steps (Coleman Flame Photometer):

1. Plug in line cord. Allow instrument to warm up at least 15 minutes before use. Where many tests are to be run, allow to run continually, turning off only the burner between tests.
2. To light flame (for natural gas):
   a. Light match.
   b. Turn on gas full, hold match inside top edge of cylindrical screen by reaching through the door opening.
   c. When yellow flame appears at top of chimney, bring oxygen pressure to about 5 lb per square inch.
   d. Check flame, if concentric, increase oxygen pressue to 13 lb per square inch. (If flame is not concentric consult book of

directions on cleaning the clogged capillary.)

e. To shut off flame, first turn off oxygen and immediately turn off gas.

3. Place filter for Na, K, or Ca in slot, name side to front.

Preparing calibration curves:

1. Set galvanometer coarse control almost to full counterclockwise position and galvanometer fine control to center, control knob in front of filter to 10.
2. Place beaker of blank solution (0.02% Sterox in distilled water) in lifter, close door and atomize. Set galvanometer reading to zero with blank control.
3. Pour standards 1, 5, 10, 25, 50 and 100 ppm into 10 ml pyrex beakers. Prepare separately the above for Na, K and $CaCO_3$.
4. Open door, replace beaker with 25 ppm Na, set galvanometer index to 65.
5. Flush atomizer with atomizing solution (0.02% Sterox) and repeat step 2.
6. Repeat step 4 and set the galvanometer to exactly 65.
7. Insert in turn the standards prepared in step 3, reading and recording each galvanometer reading for each standard from 1 to 50 ppm.
8. Clean atomizer with atomizing solution.
9. Repeat steps 4 through 9 for Na, K and $CaCO_3$ standards.
10. Prepare a calibration curve for galvanometer readings and ppm from 1 to 50 for Na, K and $CaCO_3$.
11. For higher concentration, repeat steps 4 through 6 with 100 ppm set at 65 on the galvanometer and through steps 7 and 8 with 50 ppm. Prepare a calibration curve for 50 - 100 ppm.

Procedure:

1. Plug in instrument and light flame as described above.
2. Filter water samples through Whatman No. 42 filter paper.
3. Insert Na filter.
4. Clean atomizer with atomizing solution.
5. Set galvanometer at 65 with 25 ppm Na-K-$CaCO_3$ check solution for low range; or, set at 65 with 100 ppm check solution for high range.
6. Place beaker containing sample in lifter and atomize. Read galvanometer and refer to calibration curve for ppm.
7. Clean atomizer with atomizing solution.
8. Repeat steps 6 and 7 for each sample.
9. Remove Na filter and insert K filter and repeat steps 5 through 7.
10. Remove K filter and insert Ca filter and repeat steps 5 through 7.

DICHROMATE OXIDATION ANALYSIS FOR ORGANIC MATTER
(Chemical Oxygen Demand (COD)

Total organic matter in water may be determined by evaporation to dryness, followed by determination of the loss of weight upon incineration. It may also be determined indirectly by chemical oxidation and the relationship between the average amount of oxygen required for oxidation of organic materials. One method currently in use is that for the determination of COD by oxidation with potassium permanganate. Dichromate oxidation appears superior because the reagent is not subject to autodecomposition as is true of the permanganate, and the oxidation is more complete. Permanganate oxidizes only 10 to 54 per cent of organic compounds as compared to practically 100 per cent by the dichromate method.

Boiling $K_2Cr_2O_7$ and sulfuric acid oxidizes most organic compounds with the quantitative reduction of $Cr_2O_7^{=}$ to Cr^{+++}. The excess $K_2Cr_2O_7$ can be titrated with ferrous ammonium sulfate. The reaction is:

$$6Fe(NH_4)_2(SO_4)_2 + K_2Cr_2O_7 + 7H_2SO_4 \rightarrow K_2SO_4 + 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + 6(NH_4)_2SO_4 + 7H_2O$$

Interfering Substances

Aromatic hydrocarbons and pyridine are not oxidized appreciably. Volatile compounds may be driven off by boiling before being oxidized. Chloride interfers by combining with the silver catalyst. Nitrites and Fe^{++} give high results by reducing $K_2Cr_2O_7$.

The following method is adapted from APHA Standard Methods:

Reagents:

1. Standard 1.000 N $K_2Cr_2O_7$ stock solution: Dry reagent grade $K_2Cr_2O_7$ at 103 C for 2 hours. Cool in desiccator. Dissolve 49.037 gm in distilled H_2O in 1 liter volumetric flask. Dilute to 1 liter. This is stable for several years if kept tightly stoppered.

2. Standard 0.025 N $K_2Cr_2O_7$: Dilute exactly 25.0 ml of the above standard to 1 liter in volumetric flask; add 0.1 gm sulfamic acid to eliminate interference from nitrites.

3. Sulfuric acid - silver sulfate reagent: Dissolve 22 gm Ag_2SO_4 in one 9-lb bottle of conc. H_2SO_4 (approximately 1 gm per 100 ml).

4. Ferrous ammonium sulfate: Dissolve 9.8 gm $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled H_2O, add 20 ml conc. H_2SO_4, cool, and dilute to 1 liter with distilled water. Store in brown bottle. Standardize whenever used.

5. Mercuric sulfate: Reagent grade crystals (needed only if chlorides are 200 ppm or more in sample).

6. Ferroin indicator: This reagent may be purchased or prepared as follows: dissolve 1.5 gm 1, 10-phenanthroline monohydrate and 0.7 gm $FeSO_4 \cdot 7H_2O$ in 100 ml distilled H_2O.

Special Apparatus:

A reflux apparatus using 125 or 250 ml flask with ground glass necks and Leibig or West condensers with ground glass joints must be used. Rubber or cork stoppers cannot be used since the rubber or cork will be oxidized by the dichromate. A hot plate should be used to heat the flasks.

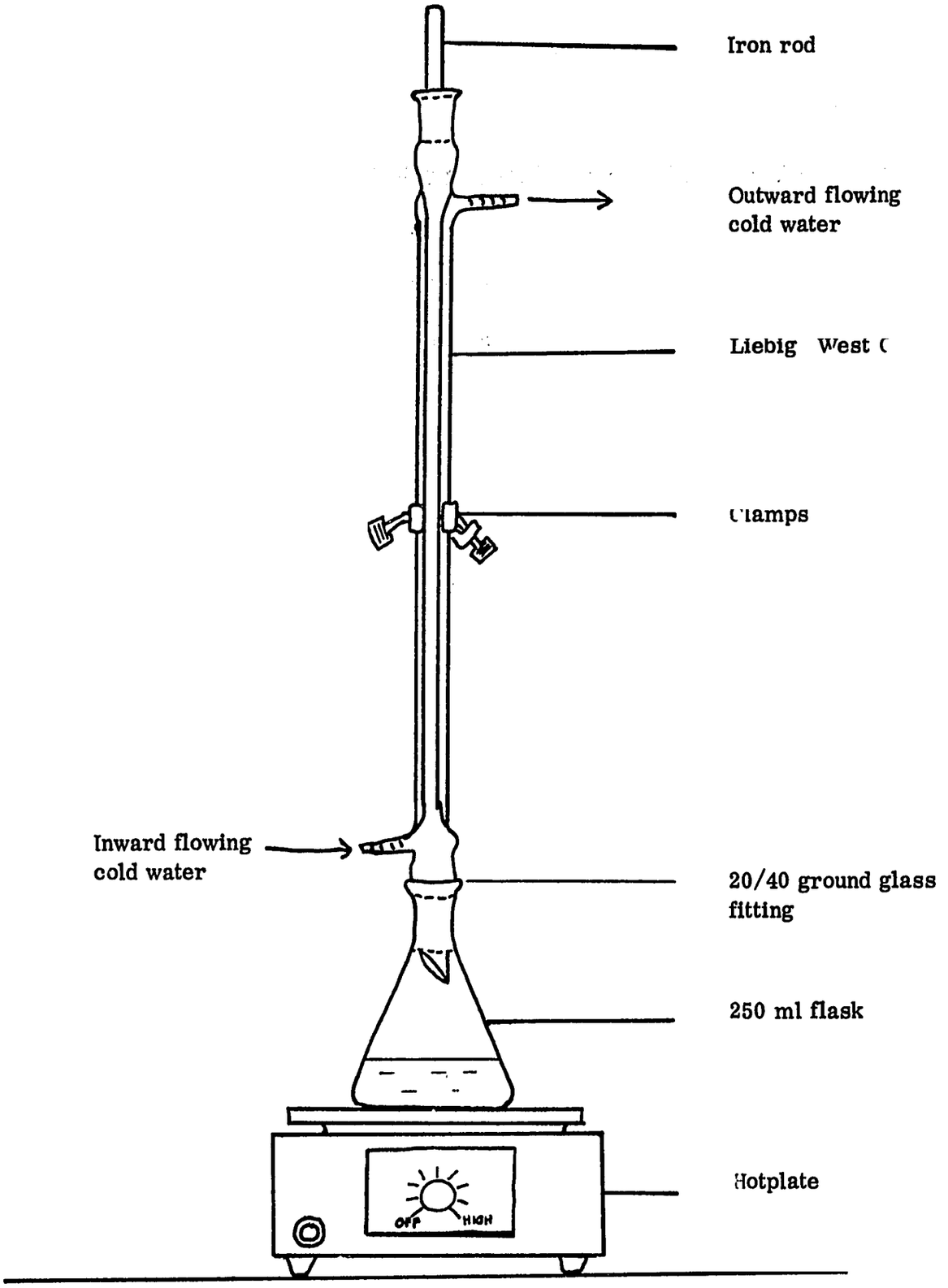


FIGURE 6. REFLEX APPARATUS FOR CHEMICAL OXYGEN DEMAND

Procedure:

1. Add 20.0 ml of sample, 0.4 gm $HgSO_4$ (if needed), 10.0 ml 0.025N $K_2Cr_2O_7$ and several glass boiling beads to reflux flask. Mix well.
2. Add carefully 30 ml sulfuric acid-silver sulfate reagent. Mix well. If the mixture is not thoroughly mixed at this point, the application of heat for refluxing may cause the mixture to be blown out the top of the condenser.
3. Reflux for 2 hours.
4. Cool, wash down condenser with 20 - 30 ml distilled water. Dilute to 75 to 100 ml.
5. Titrate excess dichromate with ferrous ammonium sulfate using 2 - 5 drops ferroin indicator. Color change is from blue-green to red-brown.
6. Carry 2 or more blanks (20 ml distilled water instead of sample) through steps 1 through 5 along with samples.

Standardization of Ferrous Ammonium Sulfate:

1. Dilute 10.0 ml 0.025N $K_2Cr_2O_7$ to about 100 ml and add 30 ml conc. H_2SO_4, allow to cool.
2. Titrate with ferrous ammonium sulfate, using 2 to 5 drops ferroin.

$$\text{Normality} = \frac{\text{ml dichromate x normality of dichromate}}{\text{ml ferrous ammonium sulfate}}$$

Standardization should be performed in duplicate.

Calculations:

$$\text{ppm COD} = \frac{(a-b)\ c \times 8000}{\text{sample vol. in ml}}$$

where a = ml Fe $(NH_4)_2(SO_4)_2$ for blank

where b = ml Fe $(NH_4)_2(SO_4)_2$ for sample

where c = normality of $Fe(NH_4)_2(SO_4)_2$

ppm organic matter = ppm COD x 0.7

The factor, 0.7, is an empirically derived average value for organic matter.

Notes

1. The glassware used must be especially clean, even a trace of organic matter can cause errors.
2. For more concentrated samples, the sample may be diluted or the $K_2Cr_2O_7$ in the reflux flask may be increased in strength. The strength of the ferrous ammonium sulfate may be increased as well to keep the titration volume within a reasonable size.
3. The normality of the $K_2Cr_2O_7$ in the reflux flask does not need to be known since it does not appear in the calculations. However, the volume and normality of the dichromate must be the same in the sample and in the blanks.

ANALYSIS OF FISH FEEDS

The elements of interest in fish feeds are protein, fat, digestible carbohydrate, indigestible fiber, and minerals. Since all determinations should be referred to the dry weight of the sample, the moisture content must also be determined. Protein, fat (ether extract), fiber, and minerals (ash) are determined directly; the remainder (protein free extract) is considered to be a measure of digestible carbohydrate, and is usually determined by difference.

Sample preparation:

The moisture content may be determined on fresh plant materials (e.g., leaves) with no preparation. Grind the dried materials from the moisture determination and use it for other determinations.

Quick-freeze fresh meat or fish samples. Within 24 hours, chop into small pieces while still frozen. Determine moisture content of frozen material. Grind the dried material and use it for other determinations.

Grind dry feeds (e.g., grains, pelleted feeds) to pass through a 20-mesh per inch screen. This material may be used for all determinations or the dried material from the moisture determination may be used for other determinations.

Moisture Content

Procedure:

1. Heat a clean porcelain crucible or dish at 100 C for 2 hours. Cool in desiccator and weigh accurately.
2. Weigh in this crucible or dish a sample of suitable size, 1 to 2 gm (or larger to provide dried material for other determinations if desired).

3. Dry at 100 C for 10 hours. Cool in desiccator and weigh. Save for ash (or other) determination.

Calculations:

$$\% \text{ moisture} = 100 \times \frac{\text{sample wt. - dry wt.}}{\text{sample wt.}}$$

If the dry residue is not used as the sample in another determination, the sample weight in that determination must be corrected for moisture. Multiply the sample weight by a factor (F) to get the dry weight of that sample.

$$F = \frac{100 - \% \text{ moisture}}{100}$$

Total Ash Content

Procedure:

1. Heat the weighed sample from step 3 of the moisture content procedure at 550 C for 4 hours.
2. Cool in desiccator and weigh.

Calculations:

$$\% \text{ ash} = \frac{\text{ash weight}}{\text{sample weight}} \times 100$$

Fat Determination (Ether Extract)

Reagents:

1. Anhydrous ether: This should be stored in tightly closed container, preferably in a refrigerator. CAUTION - highly flammable.

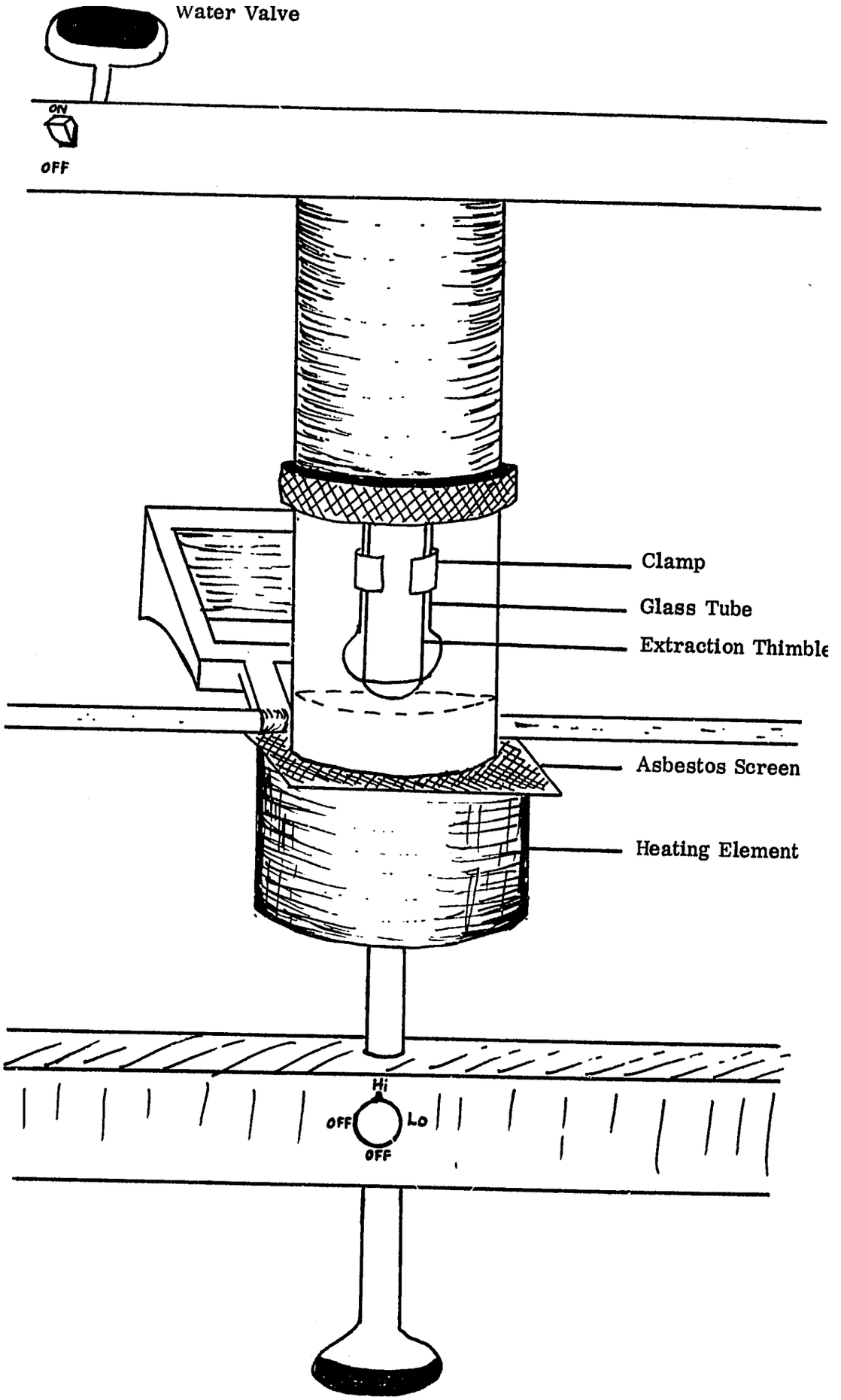


FIGURE 7. GOLDFISCH EXTRACTOR

Procedure:

1. Carefully weigh 1 to 2 gm sample of material of known moisture content and place in alundum or fiber extraction thimble*. Insert thimble into glass tube.
2. Add 35 ml anhydrous ether to a weighed beaker and attach to Goldfisch extractor.
3. Place tube containing thimble into the Goldfisch extractor. Turn on cooling water. Raise the heating elements and heat at high temperature for 4 hours. Ether should boil, but not vigorously. Turn off heater and cool.
4. Remove thimble and replace with reclaiming tube. Turn on heater and distill over most of the ether leaving fat in the beaker. Remove reclaiming tube from extractor. Turn heaters to low and let rest of ether evaporate with tilted beaker-holders down on heating elements. Too high heat may char sample.
5. Put beaker in oven at 60 - 80 C and dry for 1 hour. Cool in desiccator and weigh.
6. Save fat-free material in thimble for crude fiber or ash determination.

Calculations:

$$\% \text{ fat} = \frac{(\text{beaker} + \text{fat}) - \text{weighed beaker}}{\text{dry weight of sample}} \times 100$$

Crude Fiber

The fiber measured by this procedure is an approximation of the

*May use fiber extraction thimble if sample is not to be ashed after ether extraction. Sample can be ashed in alundum thimble following ether extraction.

indigestible matter in the feed. Accuracy and reproducibility depend on close adherence to the prescribed concentrations and timing.

Reagents:

1. Anhydrous ether: as in fat determination.
2. H_2SO_4, 1.25%: Add 7.0 ml conc. H_2SO_4 to 500 ml distilled water, mix and dilute to 1 liter.
3. NaOH, 1.25%: Dissolve 12.5 gm NaOH in distilled water and dilute to 1 liter.
4. Antifoam: This may be purchased (Dow-Corning Antifoam A is recommended) or prepared as follows: Mix 1 part paraffin oil and 1 part octyl alcohol.
5. Ethanol, 95%

Procedure:

1. Extract with ether to remove fat or use sample remaining after fat extraction.
2. Warm sample gently in hood to dive off most of the remaining ether, then dry in oven at 100 C. If sample has previously been dried for moisture determination, dry for 1 hour to drive off remaining ether; if not, dry for 10 hours to drive off moisture as well. Cool in desiccator.
3. Weigh accurately an approximately 2-gm sample. Place in 600 ml beaker.
4. Add 200 ml boiling 1.25% H_2SO_4 and 1 small drop antifoam (do not spray). Add boiling chips or beads.

5. Place beaker of hot solution on preadjusted digestion rack and boil for 30 minutes rotating beaker periodically to keep particles from adhering to sides.

6. Pour into 200 ml centrifuge tube and centrifuge for 5 minutes at 1800 rpm. Decant carefully. Add boiling water to tube until 2/3 full and stir or shake. Centrifuge and decant carefully. Repeat washing with water 3 more times.

7. Return residue to 600 ml beaker and add 200 ml boiling 1.25% NaOH and boil for 30 minutes.

8. Transfer to centrifuge tube and centrifuge and wash with boiling water as in step 6.

9. Wash the residue from centrifuge tube into 25 ml alundum crucible with boiling (80 C) ethanol, 95%. Filter by suction. Be sure all residue is in the crucible.

10. Allow the crucible to dry under a hood for a few minutes and then dry for 1 hour in a 100 C oven. Remove, cool in desiccator, weigh to get the weight of the fiber plus ash.

11. Replace crucible in muffle furnace for 3 to 5 hours at 550 to 600 C, remove, cool in desiccator, weight to get weight of ash.

Calculations:

$$\% \text{ fiber} = \frac{b - c \cdot r}{aF} \times 100$$

Where a = sample weight from step 3

Where b = weight of crucible plus sample from step 10

Where c = weight of crucible plus ash from step 11

F = factor to correct for weight of extracted fat

$$F = \frac{100}{100 - \% \text{ fat}}$$

Protein Nitrogen

The feed is digested in a Kjeldahl flask, transforming organic nitrogen to ammonium. The ammonia is distilled into a standard acid and determined by titration of the residual acid.

Reagents:

1. Potassium sulfate crystals
2. Metallic mercury
3. Concentrated H_2SO_4
4. NaOH - Na thiosulfate reagent: as in organic nitrogen analysis (page 45).
5. Metallic zinc, granular
6. 4% boric acid: Dissolve 40 gm boric acid in 1 liter.
7. Indicator solution: Dissolve 0.5 gm bromcresol green and 0.1 gm methyl red in 100 ml 95% alcohol.
8. Standard 1/14 N HCl: Prepared a stock constant boiling mixture as prepared for alkalinity test (pages 28). Dilute to 1/14 N. The volume to be diluted to 1 liter is: ml stock solution = $\frac{1/14 \times 1000}{\text{Normality of stock solution}}$ Normality of HCl may be reduced to as low as 0.01 N for samples of low protein content.

Procedure:

When feeds are to be analyzed, the following sample sizes should be taken:

(1) If a protein content of over 20% is suspected, weigh 0.7 gm samples

(2) If a protein content of under 20% is suspected, weigh 1.4 gm samples.

A reagent blank containing no sample must be analyzed at the same time.

PREPARATION OF SOIL FOR ANALYSES

Pond bottom soils to be analyzed for calcium, magnesium, inorganic phosphorus, pH, and organic matter are dried at a temperature of approximately 40 C. Drying may be done inside an oven or under an infrared lamp. To facilitate grinding, soils should be broken up into small pieces as they dry but while still moist and soft. After drying, grind soils in a mortar and sieve through a 20-mesh screen. It takes 2 to 3 days to dry 1 pint of soil sample in a suitable-sized bowl.

Soil pH

Shake 20 gm of soil sample with 20 ml distilled water for 5 minutes. Let stand 30 minutes to 1 hour. Measure pH by means of a glass electrode pH meter. While the electrodes are immersed in the mixture, swirl the mixture by rotating the container or use a mechanical stirrer. Allow 30 seconds or so for the needle to stop drifting and read while stirring.

This sample may be saved for determination of the lime requirement.

Lime Requirement of Soils*

Reagents:

1. Buffer, pH 8.00

    a. Dissolve 40 gm p-nitrophenol in approximately 350 ml hot distilled water.

*Adapted from the Auburn University Soil Testing Laboratory procedures.

b. Dissolve 30 gm boric acid in approximately 350 ml hot distilled water.

c. Dissolve 21 gm KOH pellets in approximately 25 ml distilled water.

d. Dissolve 148 gm KCl in approximately 600 ml distilled water.

e. To KCl solution add p-nitrophenol, boric acid, and KOH solutions, stirring well between each addition.

f. Dilute to 2 liters.

g. Adjust pH to exactly 8.00 with KOH or HCl as required.

Procedure:

1. Add 20 ml buffer to soil sample on which pH was determined.

2. Stir thoroughly, let stand at least 10 minutes.

3. Set pH meter at 8.00 using 1:1 buffer-water solution.

4. Read pH while stirring sample. If pH is below 7.0, dilute with 20 ml buffer and 20 ml distilled water or repeat test with 10 gm soil sample; in either of these cases, the lime requirement on the following table must be doubled.

5. Read lime requirement from Table 1.

   For example, if the soil pH is 5.2 and the pH with the buffer is 7.50, then 3500 pounds of agricultural lime is required for each acre of pond bottom to bring soil pH to 6.5

   If twice the amount of buffer, or half the amount of soil was used. then if soil pH were 5.2 and the pH after adding the buffer was 7.5, then 7000 pounds agricultural lime is required per acre of soil.

   To convert pounds/acre to kg/ha, multiply by 1.1. For example,

   3500 pounds per acre x 1.1 = 3850 kg/ha.

Lime requirement to bring soil to pH 6.5 in hundreds of pounds
Ag. lime ($CaCO_3$ x 1.5) per acre

Soil pH with buffer	Soil pH															
	6.0	5.9	5.8	5.7	5.6	5.5	5.4	5.3	5.2	5.1	5.0	4.9	4.8	4.7	4.6	4.5
7.85	6	7	7	8	9	9	10	10	11	11	12	12	12	13	13	13
7.80	8	9	10	11	11	12	13	13	14	15	15	16	16	17	17	18
7.75	10	11	12	13	14	15	16	17	18	18	19	20	20	21	21	22
7.70	11	13	15	16	17	18	19	20	21	22	23	24	24	25	26	27
7.65	13	15	17	18	20	21	22	23	25	26	27	27	28	29	30	31
7.60	15	17	19	21	23	24	26	27	28	29	30	31	32	33	34	36
7.55	17	20	22	24	26	27	29	30	32	33	34	35	36	38	39	40
7.50	19	22	24	26	28	30	32	34	35	37	38	39	41	42	43	45
7.45	21	24	27	29	31	33	35	37	38	40	41	43	45	46	47	49
7.40	23	26	29	32	34	36	38	40	42	44	46	47	49	50	52	53
7.35	25	28	31	34	37	39	41	44	45	48	51	51	53	54	56	58
7.30	27	31	34	37	40	42	45	47	49	51	53	55	57	59	60	62
7.25	29	33	36	40	43	45	48	50	53	55	57	59	61	63	65	67
7.20	31	35	39	42	45	48	51	54	56	58	61	63	65	67	69	71
7.15	33	37	41	45	48	51	54	57	60	62	64	67	69	71	73	76
7.10	34	39	44	48	51	54	58	60	63	66	68	71	73	75	78	80
7.05	36	41	46	50	54	58	61	64	67	69	72	75	77	79	82	85
7.00	38	44	48	53	57	61	64	67	70	73	76	78	81	84	86	89

Determination of Calcium in Pond Bottom Soils

Reagents

1. Extracting Solution: Ammonium Acetate - pH 7.00 To 500 ml of distilled water add 70 ml concentrated ammonium hydroxide and 60 ml concentrated acetic acid. Then add sufficient distilled water to make 1 liter. Let stand overnight and determine the pH. Adjust pH to 7.00 by adding NH_4OH or acetic acid as required.
2. Aqua-Regia Mix 3 parts conc. HCl and 1 part conc. HNO_3. Use immediately.
3. Hydrogen peroxide, reagent grade (30%).
4. HCl, concentrated.
5. NaOH solution (page 62)
6. Murexide Indicator (page 62)
7. EDTA, standard (page 60)

Procedures:

A. Preparation of the Soil Sample

1. Collect samples of bottom soils from various parts of the pond and combine to form a well-mixed composite sample.
2. Remove a half-pint sample and dry in an oven at 80 C.
3. Grind sample in mortar or in a mill until it passes a 20-mesh screen.

B. Procedure for Extraction of Ca from Soils:

1. Weigh 5.0 gm of soil into a 250 ml beaker.
2. Add 20 ml ammonium acetate extracting solution. Stir for 5 minutes.
3. Filter through a Buchner funnel using asbestos fiber. Leach further with three 10 ml portions of extracting solution.

4. Place the ammonium acetate extract in a 250 ml beaker and heat on a hot plate or steam bath under a funnel hood. Avoid boiling during evaporation. Gently heat as solution nears dryness to avoid splattering of salts. Continue heating until dense fumes of ammonium acetate have been removed.
5. Remove beakers from hot plate and add 2 to 3 ml aqua regia to dissolve salts.
6. Reheat just to dryness to remove HCl.
7. Allow beakers to cool.
8. Add 2 ml of 30% analytical grade hydrogen peroxide.
9. Warm but avoid excessive frothing. Evaporate slowly just to dryness. Do not overheat.
10. If organic matter persists after peroxide treatment (denoted by yellow color) repeat peroxide treatment as necessary.
11. Add just enough concentrated HCl to bring all calcium salts into solution. Two ml HCl is usually enough. Add a little distilled water.
12. Transfer to a 100 ml volumetric flask and make up to 100 ml with distilled water.

C. Procedure for Analysis:

1. Place a 5 ml aliquot portion of the 100 ml total in a 250 ml Erlenmeyer flask.
2. Add 45 ml distilled water.
3. Add 2 ml of approximately NaOH solution and a pinch (approximately 0.2 gm) of Murexide.

4. Titrate with standard EDTA solution.

5. Towards the end point, stop, stir or shake for about 1 minute before adding the last few drops that will determine the end point.

6. One ml of EDTA is equivalent to 1 mg $CaCO_3$. Hence, total ml of EDTA used x 20 x 200 = ppm $CaCO_3$ in soil sample.

Determination of Total Hardness of Ammonium Acetate Extract of Soils

Procedure for Analysis:

1. Place a 5 ml aliquot portion of the 100 ml total obtained in step 12 for Ca extraction from soils in a 250 ml Erlenmeyer flask.
2. Add 45 ml distilled water.
3. Add 2 ml of NH_4Cl-NH_4OH buffer.
4. Add rapidly the same amount of standard EDTA that was used for Ca analysis of this sample (page 100). Stir or shake for a short while.
5. Add 2 to 3 drops of Eriochrome black T or Calmagite (page 60).
6. Titrate to the end point.
7. One ml of EDTA is equivalent to 1 mg of $CaCO_3$. Hence, total ml of EDTA used x 20 x 200 = ppm total hardness as $CaCO_3$.

Total Phosphorus (Estimated) In Soils or Acid-NH_4F-Soluble Phosphorus

Acid-NH_4F-soluble phosphorus includes all calcium phosphates, total adsorbed phosphorus, and almost all the phosphorus held as Fe and Al complexes. Hence, subtraction of dilute-acid-soluble phosphorus and neutral-NH_4F-soluble phosphorus from acid-NH_4F-soluble phosphorus gives a difference which represents phosphorus held as Fe and Al complexes.

Reagents:

1. 0.1N HCl: Dilute 17 ml conc. HCl to 2 liters with distilled water (approximate normality only).
2. Ammonium fluoride (NH_4F). Reagent grade.

3. Ammonium molybdate-sulfuric acid solution: Same as on page 54.

4. Stannous chloride solution: Same as on page 54.

5. Boric acid: Make a saturated solution of boric acid by dissolving approximately 50 gm boric acid in 1 liter distilled water.

Procedure:

1. Shake 2 gm of soil sample with 100 ml 0.1N HCl for 30 minutes.
2. Add 2 gm of solid NH_4F. Weigh out this substance rapidly as it is hygroscopic.
3. Shake for one hour, using a mechanical shaker.
4. Filter through No. 5 Whatman filter paper.
5. To a 10 ml aliquot, add 15 ml boric acid and dilute to exactly 50 ml. Add 2 ml ammonium molybdate-sulfuric acid solution and 5 drops stannous chloride. If phosphorus concentration is high, use a 5 ml aliquot instead of 10 ml and 15 ml of distilled water instead of 10 ml. Mix well. Let stand for 10 minutes.
6. Read per cent transmittance or optical density with a spectrophotometer at 690 millimicrons, and compare with calibration curve prepared as on pages 57, 58 .
7. Multiply ppm x 250 to obtain ppm in soil.

Adsorbed Phosphorus (estimated)
or
Neutral-NH_4F-Soluble Phosphorus

Reagents:

1. Extracting solution: Dissolve 18.5 gm ammonium fluoride in 1 liter of water. Adjust the pH to 7.0 with ammonium hydroxide solution.
2. Ammonium molybdate-sulfuric acid solution: Same as on page 54 .
3. Stannous chloride solution: Same as on page 54 .
4. Boric acid: Make saturated solution of boric acid by dissolving approximately 50 gm boric acid in 1 liter of water. (Same as on page 102 .)

Procedure:

1. Shake 2 gm of soil sample with 100 ml of extracting solution for 1 hour.
2. Filter through No. 5 Whatman filter paper.
3. To a 20 ml aliquot, add 15 ml boric acid, and dilute to exactly 50 ml. Then add 2 ml molybdate solution, and 5 drops stannous chloride solution, in this order. If soil is high in phosphorus, use a 10 ml aliquot. Mix well. Let stand for 10 minutes.
4. Read per cent transmittance or optical density by means of a spectrophotometer at 690 millimicrons, and compare with the calibration curve as prepared on pages 57, 58.
5. Multiply ppm x 125 to obtain ppm in soil.

Dilute-Acid-Soluble Phosphorus (Available Phosphorus) in Soils

This inorganic phosphorus fraction of soils represents most of the phosphorus present as calcium phosphate.

Reagents:

1. Approximately 0.1N H_2SO_4. Prepare approximately 1N H_2SO_4 as described under Reagent 1, page 90. Pipette 100 ml of this reagent into a 1 liter flask and dilute to the mark with distilled water.
2. Extracting solution: In a 1 liter flask dissolve 3 gm of ammonium sulfate in 900 ml of distilled water, add 20 ml of 0.1N sulfuric acid, and dilute to 1 liter with distilled water.
3. Molybdate solution: Ammonium molybdate-sulfuric acid as prepared on page 54.
4. Stannous chloride solution: Prepared as on page 54.

Procedure:

1. Shake 2 gm of soil sample with 200 ml of extracting solution for 30 minutes
2. Filter through No. 5 Whatman filter paper.
3. Dilute a 40 ml aliquot to exactly 50 ml, add 2 ml molybdate solution and then 5 drops stannous chloride. Mix well. Let stand for 10 minutes.
4. Read per cent transmittance or optical density with a spectrophotometer at 690 millimicrons and compare with calibration chart prepared as on pages 57-58.
5. Multiply ppm x 125 to obtain ppm in soil.

Ammonia Nitrogen

Reagents:

1. Approximately 1N H_2SO_4[1]: To 800 ml distilled water in a 1 liter volumetric flask, add 27.8 ml conc. H_2SO_4 slowly, allowing it to flow down inside the flask. Mix and dilute to 1 liter.
2. Potassium sulfate-sulfuric acid solution: Dissolve 174.27 gm of pure K_2SO_4 in distilled water. Add 20 ml of approximately 1N H_2SO_4 and dilute to 2 liters. This solution contains 1N K_2SO_4 and 0.01N H_2SO_4.
3. Sodium hydroxide: Dissolve 400 gm of pure NaOH in distilled water and dilute to 1 liter. This is approximately a 10N solution.
4. Hydrochloric acid: Dilute 17 ml concentrated HCl to 2 liters with distilled water. This solution is approximately 0.1N.
5. Antifoam: Add 1 part paraffin oil to 1 part octyl-alcohol.
6. Nessler's reagent: This reagent can be purchased or made as described on page 38.
7. Standard NH_4 solution: Make as directed on page 37.

Procedure:

1. Weigh out 10 gm of air-dry soil and put into a 100 ml beaker.
2. Add 25 ml of the K_2SO_4-H_2SO_4 solution, stir to insure intimate contact, and let stand for 2 hours.

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1. Concentrated H_2SO_4 is approximately 36N, with a specific gravity of 1.84.

3. Transfer the sample to a Buchner funnel, using a #5 filter, and filter into a 250 ml Erlenmeyer flask. This procedure requires the use of vacuum apparatus.

4. Leach the sample with 125 ml additional K_2SO_4-H_2SO_4 solution, allowing the soil to drain between each addition. (Keep Soil for Organic N Analysis)

5. Dilute the filtrate to 250 ml and mix.

6. Transfer a 100 ml aliquot into a 500 ml Kjeldahl flask; the blank flask will contain 100 ml of the K_2SO_4-H_2SO_4 solution.

7. Add 100 ml distilled water, boiling chips*, 1 drop of antifoam, and 10 ml of NaOH.

8. Distill into a 250 ml Erlenmeyer flask containing 10 ml of HCl until about 150 ml of distillate is collected. (Keep Residue for NO_3+NO_2 Analysis)

9. Dilute the distillate to 500 ml with distilled water.

10. Nesslerize a 50 ml portion and evaluate ppm by spectrophotometer (pages 43-44).

11. Multiply ppm measured by 125 to obtain ppm in soil sample.

Nitrate + Nitrite Nitrogen

Reagents:

1. Anti-foam: Add 1 part paraffin oil to 1 part octyl-alcohol.

2. Devarda's alloy metal: 49.4% copper, 45.5% aluminum, and 4.7% zinc.

3. Hydrochloric acid: Dilute 17ml concentrated HCl to 2 liters with distilled water. This solution is approximately 0.1N.

4. Nessler's reagent: This reagent can be purchased or prepared as on page 38.

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*Carborundum granules, teflon chips or short sections cut from glass tubing can be used to prevent "bumping".

Procedure:

1. Add 200 ml distilled water to the residue in the Kjeldahl flasks (samples blank) left over from the NH_3 analysis.
2. Add 1 drop of anti-foam and 0.4 gm powdered Devarda's alloy metal.
3. Distill samples and blank into a 250 ml Erlenmeyer flask containing 10 ml HCl until about 125 ml of distillate is collected.
4. Dilute the distillate to 200 ml.
5. Nesslerize a 50 ml portion and evaluate by spectrophotometer (pages 43-44
6. Multiply ppm by 50 to obtain ppm in sample.

Organic Nitrogen in Pond Muds

Reagents:

1. Sulfuric acid: Concentrated H_2SO_4.
2. Sodium sulfate-mercuric oxide reagent: Mix 454 gm of Na_2SO_4 with 24.4 gm of HgO.
3. Sodium hydroxide: Prepare as described on page 106 , (approximately 10N)
4. Zinc metal: Granulated zinc.
5. Hydrochloric acid: Dilute 17 ml concentrated HCl to 2 liters with distilled water. This solution is 0.1 N.
6. Nessler's reagent: This reagent can be purchased or made as described on page 38 .

Procedure:

1. Transfer leached soils and filter papers (page 106) into 800 ml Kjeldahl flasks; the blank contains 1 No. 5 filter paper.
2. Add 40 ml H_2SO_4 and a teaspoon of Na_2SO_4-HgO reagent.

3. Digest until the residue is white (blank is clear) and an additional 10 minutes.
4. Cool, add 200 ml distilled water, mix, and cool again.
5. Add a pinch of zinc granules and 150 ml of NaOH.
6. Distill into a 250 ml Erlenmeyer flask containing 20 ml of HCl, until about 150 ml of distillate is collected.
7. Dilute the distillate to 500 ml.
8. Dilute 20 ml of the distillate to 1,000 ml.
9. Nesslerize a 50 ml portion and evaluate by spectrophotometer (pages 43-44).
10. Multiply measured ppm by 2,500 to obtain ppm in the original soil.

    10 g soil (page 106) = Distillate 500 ml (500 grams), step 7 above is a dilution of 1 to 50.

    Step 8, 20 ml to 1000 ml is a second dilution of 1 to 50.

    Total dilution = 50 x 50 = 2500

APPENDIX

SELECTED ATOMIC WEIGHTS

A	39.94	I	126.92
Ag	107.88	K	39.10
Al	26.97	Mg	24.32
As	74.91	Mn	54.93
Au	197.20	N	14.01
B	10.82	Na	23.00
Ba	137.36	O	16.00
Br	79.92	P	31.02
C	12.00	Pb	207.22
Ca	40.08	S	32.06
Cl	35.46	Se	78.96
Cu	63.57	Si	28.06
F	19.00	Sn	118.70
Fe	55.84	Sr	87.63
H	1.008	U	238.14
He	4.00	Zn	65.38
Hg	200.61		

Conversion

Centigrade to Fahrenheit Degrees

°C	°F
-18	-0.4
-16	+3.2
-14	+6.8
-12	+10.4
-10	+14.0
- 8	+17.6
- 7	+19.4
- 6	+21.2
- 5	+23.0
- 4	+24.8
- 3	+26.6
- 2	+28.4
- 1	+30.2
0	+32.0
1	33.8
2	35.6
3	37.4
4	39.2
5	41.0
6	42.8
7	44.6
8	46.4

°C	°F
9	48.2
10	50.0
11	51.8
12	53.6
13	55.4
14	57.2
15	59.0
16	60.8
17	62.6
18	64.4
19	66.2
20	68.0
21	69.8
22	71.6
23	73.4
24	75.2
25	77.0
26	78.8
27	80.6
28	82.4
29	84.2
30	86.0

°C	°F
31	87.8
32	89.6
33	91.4
34	93.2
35	95.0
36	96.8
37	98.6
38	100.4
39	102.2
40	104.0
41	105.8
42	107.6
43	109.4
44	111.2
45	113.0
46	114.8
47	116.6
48	118.4
49	120.2
50	122.0

Conversion

Grams to Pounds

Grams	Pounds	Grams	Pounds
1	0.0022	120	0.2645
2	0.0044	140	0.3086
3	0.0066	160	0.3526
4	0.0088	180	0.3967
5	0.0110	200	0.4408
6	0.0132	220	0,4849
7	0.0154	240	0.5290
8	0.0176	260	0.5730
9	0.0198	280	0.6171
10	0.0220	300	0.6612
20	0.0441	320	0.7053
30	0.0661	340	0.7494
40	0.0882	360	0.7934
50	0.1102	380	0.8375
60	0.1322	400	0.8816
70	0.1543	420	0.9257
80	0.1763	440	0.9698
90	0.1984	453.6	1.0000
100 =	0.2204		

Relation Between Transmittancy (T_s) and Optical Density (D)

T_s (%)	D	T_s (%)	D	T_s (%)	D	T_s (%)	D
100	0.000	75	0.125	50	0.301	25	0.602
99	0.004	74	0.131	49	0.310	24	0.620
98	0.009	73	0.137	48	0.319	23	0.638
97	0.013	72	0.143	47	0.328	22	0.658
96	0.018	71	0.149	46	0.337	21	0.678
95	0.022	70	0.155	45	0.347	20	0.699
94	0.027	69	0.161	44	0.357	19	0.721
93	0.032	68	0.168	43	0.367	18	0.745
92	0.036	67	0.174	42	0.377	17	0.770
91	0.041	66	0.181	41	0.387	16	0.796
90	0.046	65	0.187	40	0.398	15	0.824
89	0.051	64	0.194	39	0.409	14	0.854
88	0.056	63	0.201	38	0.420	13	0.886
87	0.061	62	0.208	37	0.432	12	0.921
86	0.066	61	0.215	36	0.444	11	0.959
85	0.071	60	0.222	35	0.456	10	1.000
84	0.076	59	0.229	34	0.469	9	1.046
83	0.081	58	0.237	33	0.482	8	1.097
82	0.086	57	0.244	32	0.495	7	1.155
81	0.092	56	0.252	31	0.509	6	1.222
80	0.097	55	0.260	30	0.523	5	1.301
79	0.102	54	0.268	29	0.538	4	1.398
78	0.108	53	0.276	28	0.552	3	1.523
77	0.114	52	0.284	27	0.569	2	1.699
76	0.119	51	0.292	26	0.585	1	2.000

STANDARDS AND CONVERSION FACTORS

Metric Prefixes

Namo	10^{-9}	used with: meter[1] (length)
Micro	10^{-6}	used with: gram (weight)
Milli	10^{-3}	used with: liter (volume), gram (weight)
Centi	10^{-2}	
Deci	10^{-1}	
Deka	10	
Hecto	10^{2}	
Kilo	10^{3}	
Mega	10^{6} (rarely used)	

Length

Inch	2.54 centimeters = 25.4 millimeters
Foot	30.48 centimeters = 0.305 meter
Yard	3 feet = 0.914 meter
Mile	5,280 feet = 1,760 yards = 1,609 kilometers = 1,609.35 meters
Fathom	6 feet = 1.829 meters
Knot	1.15 miles per hour
Micron	0.001 millimeter
Centimeter	10 millimeters = 0.39 inch = 10,000 microns
Meter	3.28 feet = 39.37 inches = 1.094 yards = 0.546 fathoms
Kilometer	3,280.83 feet = 0.621 mile

1. Micrometer = micron
   Nanometer = millimicron

Area

Square inch	6.451 square centimeters = 0.0069 square feet
Square foot	144 square inches = 0.0929 square meters
Square yards	9 square feet = 0.836 square meter
Acre	43,560 square feet = 4,840 square yards = 4,046.87 square meters = 0.405 hectare
Section	1 square mile = 640 acres = 259 ha.
Square mile	640 acres = 2.59 square kilometers = 259 ha.
Square centimeter	0.155 square inch
Square meter	10,000 square centimeters = 10.76 square feet = 2.47×10^{-4} acres
Square kilometer	247.1 acres = 1.076×10^{7} square feet = 0.386 square miles
Hectare	10,000 square meters = 2.47 acres = 100 ares
Are	0.01 hectare = 0.025 acre

Volume

Cubic inch	5.787×10^{-4} cubic feet = 16.38 ml = 0.004 gallon = 0.016 liter
Cubic foot	0.037 cubic yard = 0.028 cubic meters = 7.48 gallon = 28.32 liters
Cubic yard	27 cubic feet = 0.765 cubic meters
Cubic mile	4.165 cubic kilometers
Acre-foot	43,560 cubic feet = 1,219.68 cubic meters = 325,828.8 gallons
Minim	0.06 ml = 1/60 dram
Dram (fluid)	3.69 ml = 0.225 cubic inch = 60 minims
Teaspoon	0.33 tablespoon = 4.9 ml
Tablespoon	3 teaspoons = 14.7 ml
Ounce (fluid)	1.804 cubic inch = 29.57 ml = 0.029 liter

Cup	14.4 cubic inches = 236 ml
Pint (liquid)	28.88 cubic inches = 473.18 ml = 2 cups = 0.473 liter
Quart (liquid)	57.75 cubic inches = 0.03 cubic feet = 0.946 liter = 2 pints
Gallon	231 cubic inches = 0.134 cubic feet = 3.785 liters = 4 quarts
Bushel	1.24 cubic feet = 2,150 cubic inches = 35.24 liters
Cubic centimeter	0.061 cubic inches = 0.99997 ml
Cubic meter	35.31 cubic feet = 1.31 cubic yards = 264.17 gallons = 999.97 liters
Cubic kilometer	1×10^9 cubic meters = 0.239 cubic mile
Liter	61.03 cubic inches = 0.035 cubic foot = 0.264 gallon = 33.81 ounces (fluid)

Weight (avoirdupois)

Grain	0.065 gm
Dram	27.3 grains = 1.77 gm
Ounce	16 drams = 437.5 grains = 28.35 gm = 0.0625 pound
Pound	16 ounces = 7,000 grains = 453.59 gm = 0.454 kilogram
Ton (short)	2,000 pounds = 0.997 metric ton = 907.18 kilograms
Ton (long)	2,240 pounds = 1,016.05 kilograms

Pounds per acre x 1.12 = kilograms per hectare

Gram	0.56 dram - 0.035 ounce - 0.0022 pound
Kilogram	35.27 ounces = 2.204 pounds
Ton (metric)	2,204 pounds = 1,000 kilograms

Water

Boiling point	100 C, 212 F at 760 mm air pressure.
Freezing point	0 C, 32 F

1 Atmosphere = 33.8995 feet of water @ 4 C = 14.696 pounds square inch.

Water Pressure: (Depends upon vertical height of water column)

1 inch height @ 39.2 F (4 C) = 0.578 ounces/square inch = 5.20 pounds/square foot

1 foot height @ 39.2 F (4 C) = 0.433 pounds/square inch = 62.427 pounds/square foot

Weight of water: (Maximum density at 4 C or 39.2 F)

1 ml	1 gram @ 4 C; 0.99987 @ 0 C; 0.958 @ 100 C
1 cubic foot	62.427 pounds @ 4 C; 62.24 pounds @ 25 C
1 acre-foot	2.71×10^6 pounds(@ approximately 25 C -30 C)
1 cubic mile	3.3792×10^6 acre-feet = 9.157×10^{12} pounds = 4.579×10^9 tons
1 pint	1.04 pounds
1 quart	2.08 pounds
1 gallon	8.34 pounds
1 cubic meter	2,203.3 pounds

Density of Gases

O_2 at STP (0 C, 760 mm Hg) 1.429 gm/l

CO_2 at STP (0 C, 760 mm Hg) 1.9769 gm/l

To convert from ml gas per liter water to ppm multiply by the density of the gas at standard temperature and pressure (STP). e.g., 10 ml O_2/l x 1.429 = 14.29 ppm O_2

Alkalinity

To convert other units to ppm $CaCO_3$ or ppm CO_3^{--} multiply by the following factor:

Unit	ppm $CaCO_3$	ppm CO_3^{--}
grains $CaCO_3$/ U.S. gallon	17.1	10.25
grains $CaCO_3$/English gallon	14.3	8.58
ppm CaO	1.79	1.07
ml 0.1N HCl/100 ml sample	50	30
milliequivalent /liter	50	30

Unit	ppm $CaCO_3$	ppm CO_3^{--}
equivalent ppm	50	30
ppm $CaCO_3$	1	0.60
ppm CO_3^{--}	1.67	1