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Water Quality Analysis Laboratory Procedures Syllabus

P. A. Cowan

D. B. Porcella

V. D. Adams

L. A. Gardner

WATER QUALITY ANALYSIS LABORATORY PROCEDURES SYLLABUS

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INTRODUCTION

The 1976 edition of "Methods for Chemical Analysis of Water and Wastes" describes chemical analytical procedures to be used in Water Quality Office (WQO) laboratories. The methods were chosen through the combined efforts of the Regional Analytical Quality Control (AQC) Coordinators, Laboratory Quality Control Officers, and other senior chemists in both federal and state laboratories. Method selection was based on the following criteria:

1. The method should measure the desired constituent with precision and accuracy sufficient to meet the data needs of WQO in the presence of the interferences normally encountered in polluted waters.

2. The procedures should utilize the equipment and skills normally available in the typical water pollution control laboratory.

3. The selected methods are in use in many laboratories or have been sufficiently tested to establish their validity.

4. The methods should be sufficiently rapid to permit routine use for the examination of a large number of samples.

Except where noted under "Scope and Application" for each constituent, the methods can be used for the measurement of the indicated constituent in both water and wastewaters and in both saline and fresh water samples.

Instrumental methods have been selected in preference to manual procedures because of the improved speed, precision, and accuracy. Procedures for the Technicon AutoAnalyzer have been included for laboratories having this equipment available.

Precision and accuracy statements have been derived from interlaboratory studies conducted by the Methods and Performance Activity, Analytical Quality Control Laboratory, WQO; the American Society for Testing Materials; or the Analytical Reference Service of the Public Health Service, DHEW.

Specific instructions for the handling and preservation of samples cannot be given because of the wide variability in types of samples and local sampling situations. However, certain general principles should be followed. Wherever possible, the sampling program should be designed to provide for the shortest possible interval between sample collection and analysis. Positive steps should be taken to maintain both the concentration and the physical state of the constituents to

be measured. Where both total and dissolved concentrations are to be determined, the dissolved concentration is the amount present after filtration through a 0.45 membrane filter. When the dissolved concentration is to be determined, filtration should be carried out as soon as possible after collection of the sample, preferably in the field. Where field filtration is not practical, the sample should be filtered as soon as it is received in the laboratory.

In situations where the interval between sample collection and analysis is long enough to produce significant changes in either the concentration or the physical state of the constituent to be measured, the preservatives listed in Table 2 are recommended.

Although every effort has been made to select methods which are applicable to the widest range of sample types, significant interferences may be encountered in certain isolated samples. In these situations, the analyst should define the nature of the interference with the method herein and bring this information to the attention of the Analytical Quality Control Laboratory through the appropriate Regional AQC Coordinator. Recommendations for alternative procedures will be made and modification of the method will be developed to overcome the interferences.

SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, either domestic sewage, industrial wastes or natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source.

The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may change valence states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations may also adsorb onto surfaces (glass, plastic, quartz, etc.), such as, iron and lead. Biological changes taking place in a sample may change the state of an element or a radical to a different state. Soluble constituents may be converted to organically bound material in cell structures, or cell lysis may result in release of cellular materials into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Preservation methods are generally limited to pH control, chemical addition, refrigeration, and freezing. Table 1 shows the various preservatives that may be used to retard changes in samples.

Table 1

Preservative	Action	Applicable to:
$HgCl_2$*	Bacterial Inhibitor	Nitrogen forms, Phosphorus forms
Acid (HNO_3)	Metals solvent, prevents precipitation	Metals
Acid (H_2SO_4)	Bacterial Inhibitor	Organic samples (COD, oil & grease, organic carbon, etc.)
	Salt formation with organic bases	Ammonia, amines
Alkali (NaOH)	Salt formation with volatile compounds	Cyanides, organic acids
Refrigeration or freezing	Bacterial Inhibitor	Acidity - alkalinity, organic materials, BOD, color, odor, organic P, organic N, Carbon, etc., biological organisms (coliform, etc.)

In summary, refrigeration at temperatures near freezing or below is the best preservation technique available, but is not applicable to all types of samples.

The recommended choice of preservatives for various constituents is given is Table 2. These choices are based on the accompanying references and on information supplied by various Regional Analytical Quality Control Coordinators.

*Disposal of mercury containing samples is a recognized problem; research investigations are underway to replace it as a preservative. This text attempts to use alternative preservation techniques.

Table 2 - Sample Preservation

Parameter	Preservative	Maximum Holding Period
Acidity-Alkalinity	Refrigeration at 4°C	24 hours
Biochemical Oxygen Demand	Refrigeration at 4°C	6 hours
Calcium	None required	7 days
Chemical Oxygen Demand	2 mℓ H_2SO_4 per liter	7 days
Chloride	None required	7 days
Color	Refrigeration at 4°C	24 hours
Cyanide	NaOH to pH 10	24 hours
Dissolved Oxygen	Determine on site	No holding
Fluoride	None required	7 days
Hardness	None required	7 days
Metals, Total	5 mℓ HNO_3 per liter	6 months
Metals, Dissolved	Filtrate: 3 mℓ 1:1 HNO_3 per liter	6 months
Nitrogen, Ammonia	2 mℓ conc. H_2SO_4 per liter at 4°C	7 days
Nitrogen, Kjeldahl	2 mℓ conc. H_2SO_4 per liter at 4°C	Unstable
Nitrogen, Nitrate	1-2 mℓ chloroform per liter at 4°C	7 days
Nitrogen, Nitrite	Refrigeration at 4°C	24 hours
Oil and Grease	2 mℓ H_2SO_4 per liter at 4°C	24 hours
Organic Carbon	2 mℓ H_2SO_4 per liter (pH 2)	7 days
pH	Determine on site	No holding
Phenolics	1.0 g $CuSO_4$/ℓ + H_3PO_4 to pH 4.0 - 4°C	24 hours
Phosphorus	Refrigeration at 4°C	24 hours
Solids	None available	7 days
Specific Conductance	None required	7 days
Sulfate	Refrigeration at 4°C	7 days
Sulfide	2 mℓ Zn acetate per liter	7 days
Threshold Odor	Refrigeration at 4°C	24 hours
Turbidity	None available	7 days

References:

Jenkins, David, *A Study of Methods Suitable for the Analysis and Preservation of Phosphorus Forms in an Estuarine Environment.* Report for the Central Pacific River Basins Project, Southwest Region, FWPCA (1965).

Jenkins, David, *A Study of Method for the Analysis and Preservation of Nitrogen Forms in an Estuarine Environment.* Report for the Central Pacific River Basins Project, Southwest Region, FWPCA (1965).

Howe, L. H. and Holley, C. W., *Comparisons of Mercury (II) Chloride and Sulfuric Acid as Preservatives for Nitrogen Forms in Water Samples.* Env. Sci. & Tech. 3:478 (1969).

HOW TO WASH LABORATORY GLASSWARE

The washing of glassware frequently depends on the proposed use of the glassware. Often glassware can be color coded so that segregation for the different uses and different concentration levels can be maintained. The following list of washing methods is used at the Utah Water Research Laboratory.

A. Specific Chemical Analysis - Overnight soaking in a chromic acid solution* followed by a detergent wash and then ten rinses with tap water and three rinses with deionized water.

B. General Chemical Use - This is appropriate for most routine analyses in a teaching laboratory and the the same as Section A except that soaking in chromic acid solution is deleted.

C. Phosphorus and Nitrogen Nutrient Analyses - Wash with $NaHCO_3$ and rinse and soak overnight in 0.1 N HCl. Rinse 3 times with tap and 3 times with deionized water. Do not use detergent.

D. Biological Analyses - Wash with $NaHCO_3$ and rinse 3 times with tap and 3 times with deionized water. It may be necessary to autoclave the glassware. Do not use detergent or chromic acid soak.

* Chromic Acid Solution. Add carefully 1 liter of concentrated H_2SO_4 to 35 mℓ of a saturated solution of Na_2CrO_7.

Cleaning of Spectrophotometer Cells

1. Clean the cell with a mild agent as soon as possible after each use.
2. Always start with distilled water for aqueous solutions, or use any suitable organic solvent for organic materials.
3. Mild sulfonic detergents may be used if it is certain that they are true solutions and do not contain particulate matter.
4. For hard-to-remove deposits, use a solution of 50% 3N HCl and 50% Ethanol.
5. Whenever possilbe, rinse cell with sample solution before filling.
6. Remember that if a reagent is not of spectrograde purity it may leave a deposit on the cell window after evaporation.

NEVER blow the cell dry with air. It is better to speed evaporation of the solvent with the aid of vacuum.

NEVER use any brush or instrument which might scratch the sides of the cell.

NEVER use alkali, abrasives, etching materials, or hot concentrated acids.

NEVER use ultrasonic devices to clean cells.

SPECIAL CHEMICAL PROCEDURES

ALKALINITY

(Potentiometric)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 278-282.

2. Outline of Method: Alkalinity measures the ability of water to accept protons. The total alkalinity generally is imparted by the bicarbonate, carbonate, and hydroxide components dissolved in the water and may be measured by titration with acid. Sample should be collected in polyethylene and analyzed within 1 day.

B. SPECIAL REAGENTS

1. 0.1 N Sulfuric Acid Solution: Add exactly 2.74 mℓ of conc. H_2SO_4 (sp. gr. = 1.84) to 800 mℓ of distilled water, cool, and dilute to exactly 1 liter with DW.

2. 0.02 N H_2SO_4: Dilute 200 mℓ of 0.1 N H_2SO_4 to exactly 1 liter. This standard acid solution is equivalent to 1 mg $CaCO_3$ per 1 mℓ.

C. STANDARDIZATION

1. Dissolve exactly 1.060 g anhydrous Na_2CO_3 which has been oven dried at 140°C into distilled water and dilute to exactly 1 liter of DW. This process yields 0.02 N Na_2CO_3.

2. Take 10 mℓ of 0.02 Na_2CO_3 and dilute to 100 mℓ (do 2 replicates).

3. Titrate with 0.02 N H_2SO_4 to a 4.5 pH endpoint.

4. Calculate acid factor for the 0.02 N H_2SO_4.

$$\text{Acid Factor} = \frac{\text{mℓ } 0.02 \text{ N } Na_2CO_3}{\text{mℓ } 0.02 \text{ N } H_2SO_4} \times 0.02 \times \frac{50{,}000}{100} \approx 10$$

D. PROCEDURE

1. Predetermine total alkalinity endpoint pH:

A. Measure out 100 mℓ of sample (i.e., lake, river, etc.) into a 250 mℓ beaker and titrate using a 0.02 N H_2SO_4, adding titrant 1 mℓ at a time to a pH of about 4.5. Continuously mix on magnetic stirrer while titrating.

B. Plot results pH vs mℓ titrant. Resultant endpoint pH (about 4.5) will then be used for subsequent samples of that water type (in lake, river, etc.).

C. An example of typical results is shown as follows which can be used when time does not permit a titration curve:

GENERAL ENDPOINT CHART

Total Alk. Range mg/ℓ	pH
0-25	5.2
25-50	5.1
50-90	5.0
90-130	4.9
130-210	4.8
210-330	4.7
330-445	4.6
445-	4.5

APPROXIMATE FACTOR CHART

Sample Vol (mℓ)	Acid N	≅Factor
50	0.001	1
100	0.002	1
50	0.002	2
100	0.02	10
50	0.02	20

2. Alkalinity:

A. Measure 100 mℓ (use sample volumes requiring less than 25 mℓ titrant) into a beaker, insert bar magnet and pH meter electrodes; switch on mag-mix, making sure the bar magnet doesn't touch electrodes.

B. Record initial pH, and sample temperature.

C. If initial pH above 8.3, titrate to 8.3, recording mℓ 0.02 N H_2SO_4 used. This is the phenolphthalein endpoint.

D. Titrate to pH endpoint of 4.5, recording volume of titrant. This is the total alkalinity endpoint.

E. Calculate the total alkalinity of the sample from the total volume of the 0.02 N acid used multiplied by the corrected acid factor. This factor is determined upon each standardization of the acid.

E. EXPLANATION OF TOTAL ALKALINITY CALCULATION

I Volumetric Analysis where C = Concentration
V = Volume

$$C_s V_s = C_a V_a \qquad \text{or} \qquad C_s = \frac{C_a V_a}{V_s}$$

sample acid

$$C_a = \left(\frac{\text{mℓ 0.02 N } Na_2CO_3 \text{ Standard}}{\text{mℓ } H_2SO_4 \text{ titrant used to titrate standard}} \right) \left(0.02 \text{ N} \right)$$

Units: equiv/1

V_a = volume H_2SO_4 titrant used to titrate sample — mℓ

V_s = volume sample used, i.e., 100 mℓ — mℓ

50,000 = mg $CaCO_3$/equiv. — A factor utilized to give Total Alkalinity in terms of $CaCO_3$.

$$\text{Total Alkalinity, mg/ℓ as } CaCO_3 = \frac{\left(V_a, \text{ mℓ} \right)\left(C_a, \frac{\text{equiv}}{\ell} \right)\left(50{,}000 \frac{\text{mg } CaCO_3}{\text{equiv}} \right)}{\left(V_s, \text{ mℓ} \right)}$$

F. NOTES ON ALKALINITY COMPOSITION

Table 403:I, p. 281, *Standard Methods*, 1975.

Result of Titration	Hydroxide Alkalinity as $CaCO_3$	Carbonate Alkalinity as $CaCO_3$	Bicarbonate Alkalinity as $CaCO_3$
$P = 0$	0	0	T
$P < \frac{1}{2} T$	0	$2P$	$T - 2P$
$P = \frac{1}{2} T$	0	$2P$	0
$P > \frac{1}{2} T$	$2P - T$	$2(T - P)$	0
$P = T$	T	0	0

P - phenolphthalein alkalinity
T - total alkalinity

Conversion of total alkalinity to inorganic carbon:

1. SEE TABLE 1 (NEXT PAGE) FROM SAUNDERS, and select conversion factor from sample pH and temperature taken at time of alkalinity analysis

   mg C/ℓ = (Total Alkalinity) (Conversion Factor).

TABLE 1. Factors for the Conversion of Total Alkalinity to Milligrams of Carbon per Liter

	Temperature (°C)					
pH	0	5	10	15	20	25
5.0	9.36	8.19	7.16	6.55	6.00	5.61
5.1	7.49	6.55	5.74	5.25	4.81	4.51
5.2	6.00	5.25	4.61	4.22	3.87	3.63
5.3	4.78	4.22	3.71	3.40	3.12	2.93
5.4	3.87	3.40	3.00	2.75	2.53	2.38
5.5	3.12	2.75	2.43	2.24	2.06	1.94
5.6	2.53	2.24	1.98	1.83	1.69	1.59
5.7	2.06	1.83	1.62	1.50	1.39	1.31
5.8	1.69	1.50	1.34	1.24	1.15	1.09
5.9	1.39	1.24	1.11	1.03	0.96	0.92
6.0	1.15	1.03	0.93	0.87	0.82	0.78
6.1	0.96	0.87	0.77	0.73	0.70	0.67
6.2	0.82	0.74	0.68	0.64	0.60	0.58
6.3	0.69	0.64	0.59	0.56	0.53	0.51
6.4	0.60	0.56	0.52	0.49	0.47	0.45
6.5	0.53	0.49	0.46	0.44	0.42	0.41
6.6	0.47	0.44	0.41	0.40	0.38	0.37
6.7	0.42	0.40	0.38	0.37	0.35	0.35
6.8	0.38	0.37	0.35	0.34	0.33	0.32
6.9	0.35	0.34	0.33	0.32	0.31	0.31
7.0	0.33	0.32	0.31	0.30	0.30	0.29
7.1	0.31	0.30	0.29	0.29	0.29	0.28
7.2	0.30	0.29	0.28	0.28	0.28	0.27
7.3	0.29	0.28	0.27	0.27	0.27	0.27
7.4	0.28	0.27	0.27	0.26	0.26	0.26
7.5	0.27	0.26	0.26	0.26	0.26	0.26
7.6	0.27	0.26	0.26	0.25	0.25	0.25
7.7	0.26	0.26	0.25	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.25	0.25	0.24	0.24
8.1	0.25	0.25	0.24	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24	0.24	0.24
8.5	0.24	0.24	0.24	0.24	0.24	0.24
8.6	0.24	0.24	0.24	0.24	0.24	0.24
8.7	0.24	0.24	0.24	0.24	0.24	0.24
8.8	0.24	0.24	0.24	0.24	0.23	0.23
8.9	0.24	0.24	0.23	0.23	0.23	0.23
9.0	0.24	0.23	0.23	0.23	0.23	0.23
9.1	0.23	0.23	0.23	0.23	0.23	0.23
9.2	0.23	0.23	0.23	0.23	0.23	0.23
9.3	0.23	0.23	0.23	0.22	0.22	0.22
9.4	0.23	0.23	0.22	0.22	0.22	0.22

From: Saunders, G. W., F. B. Trama and R. W. Bachman. 1962. Evaluation of a modified ^{14}C technique for shipboard estimation of photosynthesis in large lakes. Great Lakes Res. Div., Inst. Sci. and Tech., Univ. Mich. Publ. No. 8: 1-61.

NOTES ON THE CONC. H_2SO_4: Molecular Weight = 98.08

Specific Gravity = 1.8364

% Sulfuric Acid = 97.4

Grams/liter = 1788.2

Normality = $\frac{\text{g } H_2SO_4/\text{liter}}{\text{g } H_2SO_4/\text{equiv.}}$ = 1788.2/49.04 = 36.464 N H_2SO_4

Therefore: 36.464 (mℓ) = 0.1 (1000)

mℓ = 2.74

0.1 N Sulfuric Acid is 2.74 mℓ dil. to 1 liter.

CALCIUM

(EDTA Titrimetric Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 189-190.

2. Outline of Method: Hardness in water is primarily caused by free calcium and magnesium ions, both of which are readily complexed in the presence of EDTA disodium salt. Magnesium precipitates as insoluble $Mg(OH)_2$ at pH greater than 12.0 while the calcium ions present remain in solution; therefore, at pH = 12-13, only the calcium ions present in the sample combine with the EDTA titrant. Endpoint detection is facilitated by Calver II indicator, which is red in the presence of Ca^{++} and royal blue when all of the free calcium ions have been complexed by the EDTA.

B. SPECIAL REAGENTS

1. Hydroxide Solution, 1N: Dissolve 56.1 g of KOH (or 40.00 g NaOH) in DW and dilute to 1 liter with DW.

2. Conc. EDTA disodium salt Solution, 0.01 M: Dissolve exactly 3.723 g EDTA disodium salt ($Na_2H_2C_{10}H_{12}O_8N_2 \cdot 2H_2O$) in DW and dilute to 1 liter with DW. Store in polyethylene or pyrex bottle.

3. Calver II Calcium Indicator: Hach Chemical Co., Cat No. 281.

4. Ammonium Hydroxide, 3N: Add 240 mℓ conc. NH_4OH to about 700 mℓ of DW and dilute to 1 ℓ.

C. STANDARDIZATION

Standard Calcium Solution: Weigh 1.000 g anhydrous calcium carbonate, $CaCO_3$, into a 500 mℓ Erlenmeyer flask. Using a funnel add a little at a time 1 + 1 HCl (5 mℓ HCl + 5 mℓ distilled water should make up enough) until all the $CaCO_3$ has dissolved. Add 200 mℓ distilled water and boil for a few minutes to expel CO_2. Cool, (determine pH and adjust to ~5 by adding 3 N NH_4OH or 1 + 1 HCl as required). Transfer quantitatively to a 1 liter volumetric flask and fill with distilled water.

D. PROCEDURE

1. Measure a 50 mℓ sample into a 125 mℓ Erlenmeyer flask. (Volume of the sample used for testing should be decreased to 25 mℓ and diluted to 50 mℓ with DW when the concentration exceeds 40 mg Ca^{++}/ℓ.) Avoid titer volumes > 15 mℓ.

2. Add 2 mℓ of the 1 N Hydroxide solution, or a volume sufficient to produce a pH of 12-13. Add 0.1 to 0.2 g of indicator mixture.

3. Titrate slowly with the EDTA disodium salt solution (0.01 M) until the color changes from red to blue. Wait 30-60 seconds and titrate to blue again. 1 mℓ titrant (0.0100 M EDTA) is equivalent to exactly 400.8 µg Ca^{++}. Record Sample size, dilution, and mℓ of the titer used to reach the endpoint.

E. CALCULATION

$$\text{mg/ℓ } Ca^{++} = \frac{A \times B \times 400.8}{\text{mℓ sample}}$$

Calcium Hardness as

$$\text{mg/ℓ } CaCO_3 = \frac{A \times B \times 1000}{\text{mℓ sample}}$$

where A = mℓ titration for sample
B = mg $CaCO_3$ equivalent to 1.00 mℓ EDTA titrant

Note: Because of the high pH used in this procedure, the titration should be performed immediately after the addition of the alkali.

F. NOTES

For Increased Sensitivity

1. Use 0.004 M EDTA titer.

2. Use hydroxy naphol blue indicator.

   A. 200 mg indicator/100 g NaCl.
   B. Use enough indicator to give good red color (0.2 - 0.4g).

HARDNESS

(EDTA Titrimetric Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 200-206.

2. Outline of Method: The hardness of water is due mainly to the presence of Ca^{++} and Mg^{++}. These ions form a chelated soluble complex in the presence of EDTA. Eriochrome Black T indicator is used, and as the EDTA is added, the solution will turn from wine red to blue at the endpoint. The sharpness of the endpoint is pH dependent (pH 10.0 ± 0.1). To minimize chances of $CaCO_3$ precipitation, titration should take no more than 5 minutes. The indicator functions best at room temperature.

B. SPECIAL REAGENTS

1. Buffer Solution: Dissolve 1.179 g EDTA disodium salt and 0.780 g $MgSO_4 \cdot 7H_2O$ *in* 50 mℓ distilled water. Add this solution to 16.9 g NH_4Cl and 143 mℓ conc. NH_4OH with mixing and dilute to 250 mℓ with DW. Store in tightly stoppered plastic or resistant-glass container. Stable less than 1 month.

2. Inhibitors: See *Standard Methods*.

3. Eriochrome Black T. Indicator: Mix together 0.5 g dye and 4.5 g hydroxylamine hydrochloride. Dissolve this mixture *in* 100 mℓ of 95% ethyl or isopropyl alcohol.

4. Standard EDTA Titrant, 0.01 M: See Calcium.

C. STANDARDIZATION: See Calcium

D. PROCUDURE

1. Measure a 50 mℓ sample (or a 25 mℓ aliquot diluted to 50 mℓ) into a 125 mℓ Erlenmeyer flask. Add 1-2 mℓ buffer, usually 1 mℓ buffer will be sufficient to give pH 10.0 - 10.1.

2. Add 1-2 drops of indicator and titrate slowly, stirring continuously, until the last reddish tinge disappears from the solution (adding the last few drops at 3-5 sec. intervals). 1 mℓ 0.0100 M EDTA should be equivalent to 1 mg $CaCO_3$.

E. CALCULATION

Calculate Hardness (EDTA) as

$$mg/\ell\ CaCO_3 = \frac{A \times B \times 1000}{m\ell\ sample}$$

where A = mℓ titration for sample
B = mg $CaCO_3$ equivalent to 1.00 mℓ EDTA titrant

F. NOTE

The presence of complexometrically neutral Mg in the buffer insures a good endpoint and at the same time obviates a blank correction.

ORGANIC CARBON; SOILS

A. GENERAL

1. Reference: Walkley, A. An Examination of the Methods for Determining Organic Carbon and Nitrogen in Soils. *J. Agr. Sci.* 25:598-609, 1935.

2. Outline of Method: Soils are exposed to a strong chemical oxidant under acidic conditions and the organic carbon is measured (stoichiometrically as in COD) as oxidant consumed.

B. SPECIAL REAGENTS

1. Potassium Dichromate, 1 N: Dry 53 g $K_2Cr_2O_7$ at 103°C for 2 hrs. Dissolve exactly 49.036 g of the dried crystal in distilled water and dilute to 1 ℓ. Nitrite-nitrogen exerts a COD of 1.14 mg per mg NO_2-N. Ad 0.12 g of Sulfamic Acid/ℓ of dichromate solution to eliminate the interference of nitrates up to 6 mg/ℓ.

2. Ferrous Sulfate, 1 N: Dissolve 278.02 g of $FeSO_4 \cdot 7H_2O$ in 0.5 N H_2SO_4 and dilute to 1 ℓ using the same dilute acid solution.

   a) 0.5 N H_2SO_4: 14 mℓ conc. H_2SO_4 in distilled water and diluted to 1 ℓ.

3. Ferroin Indicator
   Concentrated H_2SO_4-Ag_2SO_4 Reagent: See COD.

4. Phosphoric Acid, 85%: Conc. H_3PO_4, Analytical Reagent Grade.

C. STANDARDIZATION

1. The $FeSO_4$ was standardized each day by titrating 10.00 mℓ 1 N $K_2Cr_2O_7$ with the $FeSO_4$. 10.00 mℓ $K_2Cr_2O_7$ was drawn from a burrette into a 500 mℓ Erlenmeyer flask and 20 mℓ concentrated sulfuric acid was added. This was swirled for one minute and allowed to stand for 30 minutes to cool. About 200 mℓ of distilled water are added, followed by approx. 10 mℓ of 85 percent H_3PO_4 and 4 drops of ferroin indicator. This was then titrated with the $FeSO_4$ solution until the color changed from green to red.

NOTE 1: The stoichiometry of the reaction has been outlined by *Sawyer and McCarty* (1967). It is as follows:

$$C_nH_aO_b + cCr_2O_7^{=} + 8c\ H^+ \rightarrow nCO_2 + \frac{8c+a}{2}\ H_2O + 2c\ Cr^{3+}$$

where $c = 2/3_n + a/6 - b/3$

As can be seen, the amount of Chromate reduced per carbon atom oxidized will vary depending upon the source of the organic carbon. For instance, if $C_6H_{12}O_6$ is the carbon source 2 chromates will oxidize 3 carbons, while if C_2H_2OH is the carbon source 3 chromates will oxidize 4 carbons. It has been determined that a stoichiometry of 2 chromates for 3 carbons is an average relationship for most soils. This corresponds to a -4 change in oxidation state for each carbon and gives a milliequivalent weight for each carbon of 3 mg. Using 1 N $K_2Cr_2O_7$ this would theoretically equal 3 mg carbon oxidized for every mℓ of $K_2Cr_2O_7$ reduced.

NOTE 2: The percent recovery of the method is something that is variable from soil to soil and should be checked by a dry combustion of carbon on duplicate samples.

NOTE 3: The author found mean recoveries of about 77 percent, or a correction factor of 100/77 = 1.3:

$$\underline{1\ m\ell\ 1\ N\ K_2Cr_2O_7} = 3\ mg\ C \times 1.3 = \underline{3.9\ mg\ C}$$

D. PROCEDURE

1. An air-dried soil sample was ground in a mortar and passed through an 0.5 mm sieve.

2. 0.5 to 3.0 gram samples of the ground soil were then weighed to the nearest 0.001 gram and placed in 500 mℓ flasks. The size of the sample was chosen so as to reduce between 3.0 and 7.0 mℓ of the dichromate.

3. 10 mℓ of 1 N $K_2Cr_2O_7$ were than added from the burette to each flask containing a soil sample. To this was added 20 mℓ concentrated sulfuric acid through a quick delivery automatic pipet (which delivered the acid in less than 2 seconds).

4. Each flask was swirled for 1 minute after the addition of the acid and allowed to stand for 30 minutes.

5. About 200 mℓ distilled water are added, followed by approx. 10 mℓ 85 percent H_3PO_4 and 4 drops of ferroin indicator.

6. The samples were then titrated with the $FeSo_4$ solution until the color changed from green to red. It was always possible to determine the end-point within a fraction of a drop.

E. CALCULATION

$$\frac{\text{mg Org C}}{\text{g sample}} = \frac{(S - B) \times 3.9}{\text{g sample}}$$

S: mℓ $FeSO_4$ for Standard
B: mℓ $FeSO_4$ for Sample

FLUOROMETRIC MEASUREMENT OF "IN SITU" CHLOROPHYLL *a*

A. GENERAL

Outline of Method: Chlorophyll *a* fluoresces at the appropriate lengths and fluorescence is a more sensitive technique than turbidometric methods. Thus, a very sensitive estimate of cell biomass can be obtained by this direct and convenient technique.

B. SPECIAL APPARATUS & EQUIPMENT

1. Turner Fluorometer: Model 110
2. Cuvette: Corning #9820 rimless culture tubes
3. Lamp: Blue Lamp #110-853, T-5 envelope
4. Filters: 5-60 Primary filter for chlorophyll #110-922
   2-60 Secondary filter (substituted for 2-64)

C. STANDARDIZATION

A. Standards are made and read exactly as a sample. The resulting curves should be linear.

B. If curves not linear, check the cleanliness of the cells, or the concentration range. The readings become nonlinear using the 1x window.

D. OPERATION

A. Turn zero knob clockwise as far as possible. Check to see that the lucite button is lighted. Set sensitivity at four.

B. Allow 2 to 5 minutes warm up.

C. Check blue light source.

D. Set fluorescence dial to zero.

E. With zero knob, zero meter needle with door open.

F. Using a cell with distilled water or filtered water as a blank, insert in door and close.

G. Zero the meter needle using the blank knob.

H. Insert sample in place of blank and rotate fluorescence dial until meter needle zeroes.*

I. Read value on fluorescence dial.

J. To turn off rotate zero knob counterclockwise until lucite button is off.

E. CONCENTRATION

A. $\text{Unknown} = \frac{\text{Reading of Unknown}}{\text{Reading of Standard}} \times \text{Concentration of Standard}$

B. The window setting must be the same for both the standard and the unknown.

F. PRECAUTIONS

A. Fill cell to at least 2¼" from bottom.

B. Use distilled water or filtered sample for blank. Undistilled water has fluorescence. The use of filtered sample for a blank will correct for the background fluorescence of natural water samples.

C. The distillation apparatus should have glass or polyethylene tubing, not rubber.

D. Because system is pH sensitive, dilutions must be made with buffered solutions or filtered sample water, not distilled water.

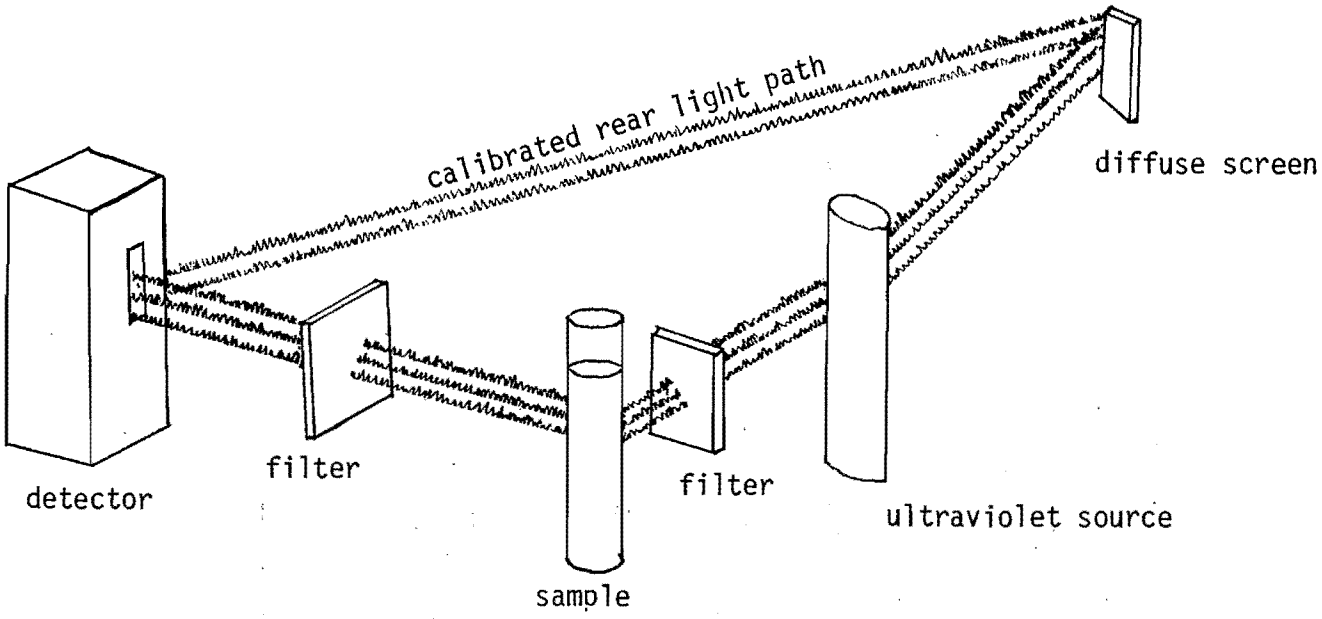
E. Standards, samples, and blanks should be at the same temperature. There may be as much as a 2 percent reduction in the reading per degree centigrade rise.

F. Cleaning is critical, use laboratory cleaners and rinse several times with distilled water. Remove fingerprints from cells.

G. The Chlorophyll *a* content of phytoplankton is variable depending on variations in light intensity, nutrient stress, and algal speciation. If samples are being prepared for each variation or 10 µm DCMU should be added to all samples and standards.

H. This method does not correct for the chlorophyll *a* degradation product (Phaeophytin *a*). If the presence of phaeophytin *a* suspected, see *Standard Methods*.

*Adjust the window between the 3x, 10x, and 30x locations until a reading is attained between 20 and 80 fluorescence units.



OPTICAL SYSTEM

References:

Slovacek, R. E., and P. J. Hannan. 1977. In Vivo Fluorescence Determinations of Phytoplankton Chlorophyll *a*. *Limn. and Ocean.* 22(5):919-924. (DCMU).

Standard Methods. 1975. pp. 1031-0133.

Strickland, J. D., and T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada. Ottawa. 310 pp.

Turner Fluorometer, Model 110, Manual.

PLANKTON PIGMENTS

(Single Transmittancy Measurements)

A. REAGENTS

1. Analytical grade acetone. 90 percent v/v aqueous solution.

2. Magnesium carbonate reagent grade fine powder.

B. PROCEDURE

Filter an adequate quantity of water (generally 1-2 liters) containing about 0.1 gram of powdered magnesium carbonate through a GF/C glass fiber filter.

To extract pigments add 5 mℓ of 90 percent acetone to a test tube or centrifuge tube, containing the folded and rolled filter. Shake and allow to stand in the dark for 18-24 hours. If immediate extraction is not practical, cover this tube containing the filter, covered with acetone to protect it from light degradation, and store it in a freezer. Samples stored in this manner should be stable for up to three months. To proceed with the extraction then, centrifuge for about 5 minutes at high speed in a clinical centrifuge. Pour off the supernatant into an optical cell of 1 cm length. Measure the transmittancies at 665, 645, and 630 mμ with a red-sensitive photocell and at 480 mμ with a blue-sensitive tube. Set the instrument against pure solvent.

C. CALCULATIONS

Concentrations of the chlorophylls may be estimated from the following equations:

$$C_a = 11.6\, A_{665} - 0.14\, A_{630} - 1.31\, A_{645}$$

$$C_b = 20.7\, A_{645} - 4.34\, A_{665} - 4.42\, A_{630}$$

$$C_c = 55\quad A_{630} - 16.3\, A_{645} - 4.64\, A_{665}$$

Where A is the absorbency at the wavelengths indicated and C_a, C_b, C_c, are the concentrations of chlorophylls a, b, and c, respectively in mg/ℓ.

The concentration of plant carotenoids may also be estimated from one of the following equations depending on which algal group predominates the plankton:

C_{nac} = 4.0 A_{480}, if crop predominately Chlorophyta or Cyanophyta.

= 10.0 A_{480}, if crop predominately Crysophyta or Pyrrophyta.

Where C_{nac} is the concentration of the non-astacin type (algal) carotenoids in m S.P.U./ℓ (milli-Specified Pigment Units).

The concentrations in a known volume of the extract can be converted to concentrations of pigments in the original sample by multiplying by the appropriate factor according to the volume of water used, the final extraction volume and the units in which the concentrations are expressed. For example, if 1.50 ℓ of water were used and if the final volume of the acetone extract prior to spectrophotometric analysis were 5.0 mℓ, multiplying by the factor 5.0/1.50 would give the concentration in mg/M^3 or µg/ℓ for chlorophylls a, b, and c, or m S.P.U./M^3 in the case of non-astacin carotenoids.

D. REFERENCES

Barnes, H. 1959. *Apparatus and methods of oceanography*. Part one: chemical. Interscience, New York. 341 p.

Creitz, G. L., and F. A. Richards. 1955. The estimation and characterization of plankton populations by pigment analyses. III. A note on the use of Millipore membrane filters in the estimation of plankton pigments. *J. Mar. Res.*, 14:211-216.

Parsons, T. R., and J. D. H. Strickland. 1963. Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *J. Mar. Res.*, 21:155-163.

Richards, F. A., and T. G. Thompson. 1952. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. *J. Mar. Res.*, 11:156-172.

Standard Methods. 1975. pp. 1029-1031.

CHLORIDE

(Mercuric Nitrate Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 302-306.

2. Outline of Method: Chloride can be titrated with mercuric nitrate because of the formation of soluble, slightly dissociated mercuric chloride. In the pH range 2.3-2.8, diphenylcarbazone indicates the endpoint of this titration by formation of a purple complex with excess mercuric ions. The indicator-acidifier automatically adjusts the pH of most potable waters to pH 2.5 ± 0.1. Xylene cyanol FF facilitates endpoint detection which goes from blue-green to blue to purple.

B. SPECIAL REAGENTS

1. Indicator-Acidifier Reagent: Dissolve in order named 0.25 g s-diphenylcarbazone, 4.0 mℓ conc. HNO_3, and 0.03 g xylene cyanol FF in 100 mℓ of 95 percent EtOH or isopropyl alcohol. Store in dark bottle in the refrigerator.

2. Conc. Mercuric Nitrate: Dissolve 2.42 g (ref. *Jenkins*) $Hg(NO_3)_2 \cdot H_2O$ in 100 mℓ DW containing 0.25 mℓ conc. HNO_3. Dilute to 1 liter with DW. Store in dark bottle. Normality = 0.0141:

   1 mℓ is equivalent to 0.50 mg Cl^-

   Standardize daily.

3. Conc. Sodium Chloride: Dissolve 0.8241 g NaCl (dried at 140°C) in DW and dilute to 1 liter. Normality = 0.0141:

   1 mℓ = 0.50 mg Cl^-

C. STANDARDIZATION

1. Measure exactly 5.0 mℓ of the conc. sodium chloride and 10 mg $NaHCO_3$ into each of 3 250 mℓ Erlenmeyer flasks. Dilute to 100 mℓ with distilled water. Pour 2 100 mℓ blanks each containing the 10 mg $NaHCO_3$.

2. Add 1.0 mℓ of the indicator-acidifier reagent; mix, and titrate with $Hg(NO_3)_2$.

$$Hg(NO_3)_2 \text{ concentration, mg } Cl^-/m\ell = \frac{2.5 \text{ mg } Cl^-}{m\ell \text{ } Hg(NO_3)_2 \text{ used for std} - m\ell \text{ } Hg(NO_3)_2 \text{ used for blank}}$$

D. PROCEDURE

1. Measure a 100 mℓ sample (or an aliquot diluted to 100 mℓ so that the chloride content is less than 10 mg) into a 250 mℓ Erlenmeyer flask.

2. Add 1 mℓ of the indicator-acidifier reagent; the pH at this point should be in the 2.3-2.8 range. At pH 2.5 ± 0.1 the indicator should give the sample a blue-green color.

3. Titrate; since the titer was standardized to a specific hue of purple, the same color must be adopted for the blanks and the samples.

E. CALCULATIONS

$$\frac{\text{mg } Cl^-}{\text{liter}} = (A\text{-}B)\ (C)\ (F)$$

where:

A = mℓ $Hg(NO_3)_2$ used to titrate the Sample

B = mℓ $Hg(NO_3)_2$ used to titrate the Blank

C = concentration of $Hg(NO_3)_2$ in mg Cl^- per mℓ $Hg(NO_3)_2$

F = factor used to convert the concentration to mg per liter

F = 10 if the sample size was 100 mℓ

F = 20 if the sample size was 50 mℓ diluted to 100 mℓ

F = 40 if the sample size was 25 mℓ diluted to 100 mℓ

F = 50 if the sample size was 20 mℓ diluted to 100 mℓ

F = 100 if the sample was 10 mℓ diluted to 100 mℓ with DW

F. NOTES:

1. HNO_3 in Indicator-Acidifier is sufficient to neutralize a total alkalinity of 150 mg/ℓ as $CaCO_3$ to the proper pH in a 100 mℓ sample. If alkaline sample, either dilute or neutralize (see *Standard Methods*).

2. $NaHCO_3$ used in blanks and standards to assure proper pH upon addition of indicator-acidifier.

RESIDUAL CHLORINE

(Iodometric Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 309-318.

2. Outline of Method: Chlorine will liberate free iodine from iodide solutions; the resultant iodine is then titrated with a standardized thiosulfate solution. A pH range of 3 to 4 is preferable. Minimum detectable range with 0.01 N titer is 40 µg/ℓ Cl. Chlorine in aqueous solutions - not stable... do not store!

B. SPECIAL REAGENTS

1. Acetic Acid: conc. (glacial)

2. Potassium Iodide: crystals

3. Stock Std. Sodium Thiosulfate, 0.1 N: Dissolve 24.82 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter of freshly boiled distilled water and standardize after at least 2 weeks storage. Add a few mℓs of chloroform to minimize bacterial decomposition of solution.

4. Dilute Sodium Thiosulfate, 0.01 N: Dilute aged stock using freshly boiled distilled water.

5. Starch: See Dissolved Oxygen.

6. Stock Std. Potassium Dichromate, 0.100 N: Dissolve 4.904 g of anhydrous $K_2Cr_2O_7$ (dried at 103°C for 1 hour) in distilled water and dilute to 1 liter. Store in glass stoppered bottle.

C. STANDARDIZATION

Use 0.100 N dichromate to standardize 0.1 N thiosulfate and 0.0100 N dichromate for 0.01 N thiosulfate.

1. To 80 mℓ distilled water, add with constant stirring, 1 mℓ conc H_2SO_4, 10.00 mℓ dichromate solution, and 1 g of KI. Allow mixture to stand for 6 min. in the dark before titrating.

FOR 0.1 N $Na_2S_2O_3$ $N = \dfrac{1.0}{\text{mℓ } Na_2S_2O_3 \text{ consumed}}$

FOR 0.01 N $Na_2S_2O_3$ $N = \dfrac{0.1}{\text{mℓ } Na_2S_2O_3 \text{ consumed}}$

D. PROCEDURE

Cl	Sample Size, mℓ
< 1 mg/ℓ	1000
1-10 mg/ℓ	500
> 10 mg/ℓ	proportionally less sample

1. Place 5 mℓ glacial acetic acid (or sufficient to reduce pH to between 3 and 4) in a flask, add 1 g KI (estimate).

2. Pour in sample and mix.

3. Titrate immediately with standardized thiosulfate (away from direct sunlight) adding starch just prior to endpoint.

4. Run a blank of distilled water by taking a volume of distilled water corresponding to the sample used, add 5 mℓ glacial, 1 g KI, and 1 mℓ starch.

BLANK TITRATION

A. IF a blue color occurs:
titrate to clear and subtract this amount from sample titration.

B. IF no blue color occurs:
see *Standard Methods*.

E. CALCULATIONS - Total Available Residual Chlorine

$$\text{mg/ℓ Cl} = \frac{(A - B)\ N\ (35{,}450)}{\text{mℓ sample}}$$

A = mℓ titration for sample
B = mℓ titration for blank
N = $Na_2S_2O_3$ Normality

Conversion Factor (35,450) = mg Cl/eq. Cl

RESIDUAL CHLORINE

(Amperometric Titration Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 322 - 325.

2. Outline of Method: Free available chlorine is titrated with standardized phenylarsin oxide titrant using amperometric detection at a pH between 6.5 and 7.5, a range in which the combined chlorine reacts slowly. The combined chlorine, in turn, is titrated in the presence of the proper amount of potassium iodide in the pH range 3.5 - 4.5.

B. SPECIAL REAGENTS

1. Standard Phenylarsine Oxide: Dissolve ~0.8 g phenylarsin oxide powder, C_6H_5AsO, in 150 mℓ 0.3 N sodium hydroxide. After settling, decant 110 mℓ of this solution into 800 mℓ distilled water and mix thoroughly. Bring the solution to pH 6-7 with 6 N HCl solution and finally dilute to almost 1 l. Caution: toxic - take care to avoid ingestion.

2. Standard Sodium Arsenite, 0.1 N: Accurately weigh a stoppered weighing bottle containing ~4.95 g arsenic trioxide, As_2O_3, primary standard grade. Transfer without loss to a 1-l volumetric flask and again weigh the bottle. Do not attempt to brush out the adhering oxide. Moisten the As_2O_3 with distilled water and add 15 g NaOH and 100 mℓ distilled water. Swirl contents of the flask gently until the As_2O_3 is in solution. Dilute to 250 mℓ with distilled water and saturate the solution with CO_2, thus converting all the NaOH to sodium bicarbonate. Dilute to the mark, stopper the flask and mix thoroughly. Caution: toxic - take care to avoid ingestion.

3. Standard Iodine, 0.1 N: Dissolve 40 g KI in 25 mℓ distilled water and then add 13 g resublimed I_2 and stir until dissolved. Transfer to a 1 l volumetric flask and dilute to the mark.

4. Diluted Standard Iodine, 0.0282 N: Dissolve 25 g KI in a little distilled water in a 1 l volumetric flask, add the proper amount of 0.1 N iodine solution exactly standardized to yield a 0.0282 N solution and dilute to 1 l. Store in a brown bottle (keep from all contact with rubber) in the dark and standardize daily.

5. Phosphate Buffer Solution, pH 7: Dissolve 25.4 g anhydrous potassium dihydrogen phosphate, KH_2PO_3, and 34.1 g anhydrous disodium hydrogen phosphate, Na_2HPO_4, in 800 mℓ distilled water. Add 2 mℓ sodium hydrochlorite solution containing 1 percent available chlorine and mix thoroughly. Protect from sunlight for several days and then expose to sunlight until no residual chlorine remains. If necessary use sodium sulfite for final dechlorination. Finally dilute to 1 l with distilled water and filter if necessary.

6. Potassium Iodide Solution: Dissolve 50 g KI and dilute to 1 l, using freshly boiled and cooled distilled water. Store in a brown glass stoppered bottle, in the refrigerator.

7. Acetate Buffer Solution, pH 4: Dissolve 480 g conc. (glacial) acetic acid and 243 g sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$, in 400 mℓ distilled water and dilute to 1 l.

C. STANDARDIZATION

$$\text{Normality of sodium arsenite} = \frac{\text{g } As_2O_3}{49.445}$$

D. PROCEDURE

Sample Volume:

Residual chlorine	Sample Size, mℓ
2 mg/ℓ or less	200
>2 mg/ℓ	100 or proportionately less

Sample volume such that no more than 2 mℓ of phenylarsine oxide titrant is necessary.

Free Available Chlorine:

1. If the pH of the sample is not between 6.5 and 7.5 add 1 mℓ pH 7 phosphate buffer solution to produce a pH of 6.5-7.5.

2. Titrate with standard phenylarsine oxide observing the current changes on the microammeter. First adjust meter until needle is about in middle of range. For each increment PAO added - the needle makes a certain increment to the left. When the needle moves a smaller increment for the same increment of PAO, the end point has been reached. This can be verified by adding another increment of PAO.

Combined Available Chlorine:

1. To the sample remaining from the free - chlorine titration add 1 mℓ KI solution and then 1 mℓ acetate buffer solution, in that order.

2. Titrate with phenylarsine oxide titrant to an end point, as above.

E. CALCULATIONS

Convert the individual titrations for free available and combined available chlorine into mg/ℓ by the following equation:

$$\text{mg/ℓ Cl} = \frac{A \times 200}{\text{mℓ sample}}$$

where A = mℓ phenylarsine oxide titrant.

Standard iodine, 0.1 N

Accurately measure 40-50 mℓ of 0.1 N sodium arsenite solution into a flask and titrate with the 0.1 N iodine solution, using starch solution as indicator. Accurate results are only obtained if the solution is saturate with CO_2. This can be done by bubbling CO_2 through the solution or by adding acid (dilute HCl) near the end point.

Standard Phenylarsine Oxide Titrant:

Determine the normality of phenylarsine oxide solution by amperometric titration of standard 0.0282 N iodine solution. Adjust the final concentration of phenylarsine oxide to the desired 0.00564 N before completing the final standardization with the amperometric titrator (*i.e.* 5 mℓ PAO = 1 mℓ I_2).

1.00 mℓ of exactly 0.00564 N phenylarsine Oxide.

Titrant = 200 mg available chlorine. If you use 200 mℓ sample (marked on titrator), 1 mℓ PAO = 1 mg/ℓ chlorine.

(Preserve with 1 mℓ chloroform).

TOTAL COLIFORM

(Membrane Filter Technique)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 928-935.

2. Outline of Method: Sample is obtained in a sterile bottle or plastic bag and is filtered through a sterile 0.45 μ Millipore filter, which is placed on a sterile pad saturated with liquid media. The filter is then incubated at 35 ± 0.5°C for 22-24 hours. If Drinking Water Standard is required, an enrichment step should be included.

B. MATERIALS AND CULTURE MEDIA

1. Sample Bottles: Pyrex glass wide mouth bottle with rubber lined cap, approx. 125 mℓ capacity. Whirl-Pak bags are acceptable. For larger filtration volumes, any screw cap bottle capable of being sterilized is acceptable.

2. Petri Dishes: 50 mm, disposal plastic, tight sealing. 90 percent relative humidity must be maintained inside the dish. Humidity may be maintained by incubation inside any container in which the atmosphere is saturated with water.

3. Pipets and Pipet Containers: Pipets can be wrapped in paper for sterilization when aluminum or stainless steel containers are not available. Bacteriological-Serological graduated 1 and 10 mℓ pipets are recommended.

4. Filtration Membranes: Standard Millipore filter holder assembly (glass, porcelain or any noncorrosive bacteriologically inert metal) with side arm filtration flask.

5. Filter Membranes: 0.45 μ Millipore filters.

6. Absorbent Pads: Millipore.

7. Forceps: Nonserated with round or blunt tips.

8. Microscope: A binocular wide field dissecting scope; a small fluorescent lamp with magnifier is acceptable. Optical system with an incandescent light source is unsatisfactory.

9. Culture Media: M-Endo Broth MF. Dissolve 4.8 g in 100 mℓ distilled water which contains 2 mℓ of ethanol. Heat to boiling Don't prolong boiling or heat in autoclave. Cool to room

temperature, covered with foil. M-Endo Agar LES may also be used.

10. Enrichment Culture Media: Lauryl Sulfate Broth. Dissolve 35.6 g of the powder in 1000 mℓ of distilled water. Sterilize by autoclaving at 121°C (15-17 psi) for 35 min.

11. Buffer Stock: Dissolve 24.0 g KH_2PO_4 in 500 mℓ distilled water, adjust pH to 7.2 with 1 N NaOH and dilute to 1 liter with distilled water.

12. Dilution Water: Add 1.25 mℓ stock buffer and 1 g Bacto Peptone to 1 liter distilled water. Sterilize by autoclaving for 35 min. at 121°C (15-17 psi).

13. Sterilization: Sample bottles and Petri dishes (other than plastic), filtration units, membrane filters and absorbent pads are sterilized in an autoclave at 121°C (15-17 psi) for 15 min. Enrichment broth and dilution water may be sterilized at the same time. Pipets and containers are sterilized in an oven at 170°C for 2 hours. Filtration units may be sterilized by placing them in a UV cabinet for 2 min.

    CAUTION: Always use slow exhaust when liquids are included in the material to be sterilized in the autoclave.

14. Sampling: Collect sample in a sterile bottle such that water doesn't flow across hand and into the bottle. In a moving stream, point opening of bottle upstream and sweep bottle through water against the current, again, so there is no hand contamination.

D. PROCEDURE

A. Alcohol-flame forceps prior to each handling of filters or pads. Touch only the edge of the filters with the forceps.

B. Select sample size based upon expected bacterial density, about 50 coliform colonies and not more than 200 colonies of all types. If less than 20 mℓ of sample is filtered, a small amount of sterile dilution water should be added to the funnel before filtration.

    1. Place pad in Petri dish bottom (smaller diameter half) and pipet 1.8-2.0 mℓ of M-Endo Broth MF on absorbent pad. (Enough broth to saturate the pad. Excess media may be poured out of the dish.)

    2. Place membrane filter in filtration apparatus and filter vigorously shaken sample under partial vacuum. Rinse filter using three 20-30 mℓ portions of sterile dilution water.

NOTE: Use one sterile filtration apparatus for each sample (dilution series of the same sample).
Dilution series: filter in order from highest dilution (smallest amount of sample) to lowest dilution (greatest amount of sample).

3. Remove membrane filter from filtration unit and roll it on the pad avoiding any entrapment of air.

4. Incubate for 22-24 hrs. at 35 ±0.5°C.

5. Count typical coliform colonies, those which have a pink to dark red color with a metallic surface sheen.

6. Compute coliform densities from the membrane filter count within the 20-80 coliform colony range.

E. CALCULATION:

$$\text{TOTAL COLIFORM/100 m}\ell = \frac{\text{COLIFORM COLONIES COUNTED} \times 100}{\text{m}\ell \text{ sample filtered}}$$

F. ENRICHMENT PROCEDURE

1. Filter sample as above.

2. Place pad on Petri dish top (larger diameter half) and pipet 1.8-2.0 mℓ of enrichment media on the pad. (Enough media to saturate the pad with no excess.)

3. Remove membrane filter from filtration unit and roll it on the pad avoiding any entrapment of air.

4. Incubate the filter, without inverting the dish, for 1½-2 hrs. at 35 ± 0.5°C in an atmosphere of at least 90 percent relative humidity.

5. Remove from incubator and separate dish halves. Place a fresh sterile pad on the bottom half of the Petri dish and saturate it with 1.8-2.0 mℓ of M-Endo Broth MF.

6. Roll membrane filter off enrichment pad (discard old pad) and roll it on the new M-Endo pad.

7. Incubate the dish, inverted, for 20-22 hrs. at 35 ± 0.5°C.

8. Count and compute as above.

NOTES:

1. Sterilization of all equipment is essential. Buy either sterile plastic or autoclavable glass Petri dishes.

2. After tests have been completed, disposabe Petri dishes are autoclaved at 121°C (15-17 psi) for 15 min. This will destroy the dish (melts) and any bacteria present. Remains may than be thrown away.

FECAL COLIFORM

(Membrane Filter Technique)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 937-939.

2. Outline of Method: Samples are collected and filtered through a 0.45 μ Millipore filter which is cultured on an M-FC saturated pad for 24 hrs. at 44.5 ± 0.2°C. This procedure yields 93 percent accuracy for culturing coliform bacteria which originate in warm-blooded animals; method selectivity is due to the high incubation temperature.

B. MATERIALS AND CULTURE MEDIA

1. Culture Dishes: Tight fitting plastic dishes approximately 50 mm diameter.

2. Plastic Bags: Whirl-Pak bags.

3. Incubator: Stirring water bath capable of 44.5 ± 0.2°C temperature tolerance.

4. M-FC Broth: Dissolve 3.7 g of broth in 100 mℓ distilled water. Add 1.0 mℓ of 1 percent Rosolic Acid in 0.2 N NaOH. Heat to boiling, promptly remove from heat and cool to below 45°C. DO NOT AUTOCLAVE. Media can be stored at 2-10°C and unused portion must be discarded 96 hrs. after preparation.

5. Filtration Set-up, 0.45 μ MF, pads, pipets and pipet containers, dilution water and forceps: See Total Coliform.

6. Sample bottles and Petri dishes (other than plastic), filtration units, membrane filters and absorbent pads are sterilized in an autoclave at 121°C (15-17 psi) for 15 min. Pipets and containers are sterilized in an oven at 170°C for 2 hours. Filtration units may be sterilized by placing them in a UV cabinet for 2 min.

   CAUTION: Always use slow exhaust when liquids are included in the material to be sterilized in the autoclave.

7. Sampling: Collect sample in a sterile bottle such that water doesn't flow across hand and into the bottle. In a moving stream, point opening of bottle upstream and sweep bottle through water against the current, again, so there is no hand contamination.

D. PROCEDURE

A. Alcohol-flame orceps rior to each handling of filters or pads. Touch only the edge of the filters with the forceps.

B. Sample size is selected depending upon bacterial densities. Samples yielding colony counts of between 20 and 60 fecal coliform colonies give highest accuracy. If less than 20 mℓ of sample is filtered, a small amount of sterile dilution water should be added to funnel before filtration.

1. Place absorbent pad in Petri dish and pipet approximately 2 mℓ of M-FC medium to saturate the pad. Excess media may be poured out of the dish.

2. Filter as in Total Coliform.

3. Roll membrane filter on pad being careful to exclude air bubbles.

4. Wrap 4 to 6 dishes in a Whirl-Pak bag and anchor below the water surface with dishes inverted and horizontal. (Well below surface to maintain critical temperature requirements.)

5. Incubate in water bath for 24 hours at 44.5 ± 0.2°C.

6. Count colonies produced by fecal coliform bacteria; they are blue in color. Nonfecal coliform colonies are gray to cream color. Desired fecal coliform range: 20-60 colonies per filter.

E. CALCULATION

$$\text{Fecal Coliform Bacteria/100 m}\ell = \frac{\text{fecal coliform colonies counted} \times 100}{\text{m}\ell \text{ sample filtered}}$$

F. NOTES

1. Sterilization of all equipment is essential. Buy either sterile plastic or autoclavable glass Petri dishes.

2. After tests have been completed, disposable Petri dishes are autoclaved at 121°C (15-17 psi) for 15 min. This will destroy the dish (melts) and any bacteria present. Remains may then be thrown away.

SPECIFIC CONDUCTANCE

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 71-75

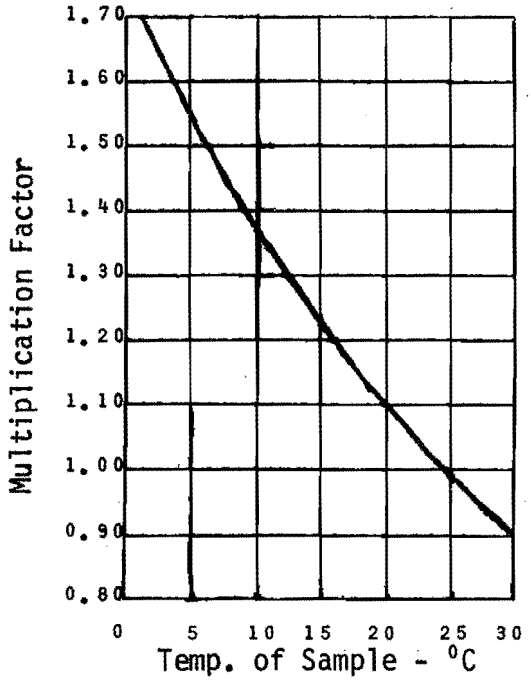
2. Outline of Method: Specific conductance measures water's capacity to carry an electric current. This property is related to the total concentration of the ionized substances in the water and the temperature at which the measurement is made. Specific conductance is used to monitor the quality of deionized water. The amount of dissolved ionic matter in a sample can be estimated by multiplying the conductivity by some empirical factor.

B. SPECIAL REAGENTS

Standard Potassium Chloride, 0.0100 M: Dissolve 745.6 mg anhydrous KCl in freshly boiled distilled water and dilute to 1 ℓ. At 25°C, this solution has a specific conductance of 1413 µmhos/cm. Store in glass stoppered pyrex bottle.

C. STANDARDIZATION

Measure temperature and *EC* of standard solution, C_{std}.



D. PROCEDURE

1. Assemble conductivity meter, rinse the electrode thoroughly with distilled water.

2. Measure temperature and *EC* of samples, C_{sam}.

E. CALCULATIONS:

1. If temperatures of the samples and the standard are the same,

$$\text{Specific Conductance} = \frac{1413}{C_{std}} \cdot C_{sam} \text{ µmhos/c}$$

2. If temperatures are different, correct all readings to 25°C (see table next page), and then calculate specific conductance by the above formula.

FACTORS FOR CONVERTING SPECIFIC CONDUCTANCE OF WATER TO EQUIVALENT VALUES AT 25°C.*

$$EC_{25}=EC_t \times f_t;\ EC_{25}=(k/R_t) \times f_t;\ R_{25}=R_t/f_t$$

°C.	°F.	f_t	°C.	°F.	f_t	°C.	°F.	f_t
3.0	37.4	1.709	22.0	71.6	1.064	29.0	84.2	0.925
4.0	39.2	1.660	22.2	72.0	1.060	29.2	84.6	.921
5.0	41.0	1.613	22.4	72.3	1.055	29.4	84.9	.918
6.0	42.8	1.569	22.6	72.7	1.051	29.6	85.3	.914
7.0	44.6	1.528	22.8	73.0	1.047	29.8	85.6	.911
8.0	46.4	1.488	23.0	73.4	1.043	30.0	86.0	.907
9.0	48.2	1.448	23.2	73.8	1.038	30.2	86.4	.904
10.0	50.0	1.411	23.4	74.1	1.034	30.4	86.7	.901
11.0	51.8	1.375	23.6	74.5	1.029	30.6	87.1	.897
12.0	53.6	1.341	23.8	74.8	1.025	30.8	87.4	.894
13.0	55.4	1.309	24.0	75.2	1.020	31.0	87.8	.890
14.0	57.2	1.277	24.2	75.6	1.016	31.2	88.2	.887
15.0	59.0	1.247	24.4	75.9	1.012	31.4	88.5	.884
16.0	60.8	1.218	24.6	76.3	1.008	31.6	88.9	.880
17.0	62.6	1.189	24.8	76.6	1.004	31.8	89.2	.877
18.0	64.4	1.163	25.0	77.0	1.000	32.0	89.6	.873
18.2	64.8	1.157	25.2	77.4	.996	32.2	90.0	.870
18.4	65.1	1.152	25.4	77.7	.992	32.4	90.3	.867
18.6	65.5	1.147	25.6	78.1	.988	32.6	90.7	.864
18.8	65.8	1.142	25.8	78.5	.983	32.8	91.0	.861
19.0	66.2	1.136	26.0	78.8	.979	33.0	91.4	.858
19.2	66.6	1.131	26.2	79.2	.975	34.0	93.2	.843
19.4	66.9	1.127	26.4	79.5	.971	35.0	95.0	.829
19.6	67.3	1.122	26.6	79.9	.967	36.0	96.8	.815
19.8	67.6	1.117	26.8	80.2	.964	37.0	98.6	.801
20.0	68.0	1.112	27.0	80.6	.960	38.0	100.2	.788
20.2	68.4	1.107	27.2	81.0	.956	39.0	102.2	.775
20.4	68.7	1.102	27.4	81.3	.953	40.0	104.0	.763
20.6	69.1	1.097	27.6	81.7	.950	41.0	105.8	.750
20.8	69.4	1.092	27.8	82.0	.947	42.0	107.6	.739
21.0	69.8	1.087	28.0	82.4	.943	43.0	109.4	.727
21.2	70.2	1.082	28.2	82.8	.940	44.0	111.2	.716
21.4	70.5	1.078	28.4	83.1	.936	45.0	113.0	.705
21.6	70.9	1.073	28.6	83.5	.932	46.0	114.8	.694
21.8	71.2	1.068	28.8	83.8	.929	47.0	116.6	.683

* From Agriculture Handbook 60, U.S.D.A.

DISSOLVED OXYGEN

(Winkler with Azide Modification)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 443-449.

2. Outline of Method: A divalent manganese solution, followed by a strong alkali, is added to the sample. Any dissolved oxygen rapidly oxidizes an equivalent amount of divalent manganese to basic hydroxides of higher valency states. When the solution is acidified in the presence of iodide, the oxidized manganese again reverts to the divalent state and iodine, equivalent to the original dissolved oxygen content of the water, is liberated. The amount of iodine is then determined by titration with standardized thiosulfate solution.

B. SPECIAL REAGENTS

1. Manganese Sulfate Solution: Dissolve 364 g $MnSO_4 \cdot H_2O$ in DW and dilute to 1 liter; filtration of the reagent may be necessary if dissolution is not complete.

2. Alkali-iodide-azide Reagent: Dissolve 500 g of solid NaOH and 135 g NaI (sodium Iodide) in DW and dilute to 1 liter. Add to this solution, 10 g NaN_3 (sodium azide) dissolved in 40 mℓ DW.

3. Conc. Sulfuric Acid: About 36 N H_2SO_4. Hence, 1 mℓ is equivalent to about 3 mℓ of the alkali-iodide-azide reagent.

4. Starch Solution: Prepare an emulsion of starch by grinding 2.5 g of soluble starch and a few mℓ of DW in a mortar. Pour this emulsion into 400 mℓ of boiling DW. Dilute to 500 mℓ. Allow to boil a few minutes; let settle overnight. Use the clear supernate. Store in a plastic squeeze bottle in the refrigerator. Stable 1 month; discard the solution when the endpoint color is no longer pure blue but takes on a green or brown tint. This solution may be preserved with 1.25 g salicylic acid per liter or by the addition of a few drops of toluene.

5. Sodium Thiosulfate Stock Solution, 0.10 N: Dissolve 24.82 g $Na_2S_2O_3 \cdot H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 mℓ chloroform or 1 g NaOH per liter.

6. Standard $Na_2S_2O_3$ Titrant, 0.025 N: Dilute 250 mℓ stock to 1 liter; exactly 1 mℓ 0.0250 N is equivalent to 200 g D.O.

7. Standard Potassium Dichromate, 0.025 N: Dry approximately 2-3 g $K_2Cr_2O_7$ at 103°C for 2 hours and then dissolve 1.226 g of the dried $K_2Cr_2O_7$ in distilled water and dilute to 1 liter.

C. STANDARDIZATION

Thiosulfate Standardization: Dissolve approximately 2 g KI (free of iodate) in 150 mℓ distilled water in a 500 mℓ Erlenmeyer flask. Add 10 mℓ of 1 + 9 H_2SO_4 followed by exactly 20 mℓ of Standard 0.025 N $K_2Cr_2O_7$. Place in dark for 5 min., dilute to approximately 400 mℓ and titrate with 0.025 N thiodulfate solution

$$N \text{ of } N_2S_2O_3 = \frac{(0.025)\ (2)}{\text{mℓ } Na_2S_2O_3 \text{ used}}$$

D. PROCEDURE

1. Rinse a 300 mℓ BOD bottle with sample. Pour the sample into the BOD bottle using a reversing sampler with a length of rubber tubing which extends from the top to the bottom of the bottle. The end of the rubber tube must remain beneath the surface of the water as the bottle is filled. Water is allowed to overflow from the top of the bottle (at least 1/3 of the volume of the bottle should be allowed to overflow). The bottle is then stoppered when all the air bubbles, if any, have been allowed to rise out of the BOD bottle. Temperature of sample should be recorded.

2. Remove the glass stopper and add 2 mℓ of $MnSO_4$ reagent followed by 2 mℓ of the alkali-iodide-azide reagent; introduce both these reagents beneath the surface of the sample. Replace the stopper being careful not to trap air inside. Mix by inverting bottle at least 15 times. Allow floc to settle, shake again. Allow floc to settle again and remove the stopper and immediately add 2 mℓ conc. H_2SO_4 by allowing acid to run down neck of bottle, restopper and mix until the precipitate dissolves leaving a clear yellow orange iodine solution. Dissolution should be complete. Samples stored at this point should be protected from strong sunlight and titrated as soon as possible (within 4 to 8 hrs).

3. Measure 203 mℓ of sample (this corresponds to 200 mℓ of original sample) into a 250 mℓ Erlenmeyer flask.

4. Rinse the burette with fresh 0.025 N $Na_2S_2O_3$ and then titrate to a faint yellow color (use a white background). Add 1-2 mℓ of the starch solution and continue the titration until the solution changes from blue to clear.

Note: This titration must not be delayed and the thiosulfate should be added fairly rapidly. Solutions should remain colorless for at least 20 seconds at the endpoint.

Note: Use of the starch solution facilitates clear endpoint detection by forming a blue complex with any iodine remaining in the solution.

5. 1 mℓ of 0.025 N $Na_2S_2O_3$ is equivalent to 200 μg D.O.; therefore, if a 203 mℓ sample (200 mℓ of original sample) is titrated, 1 mℓ 0.025 N $Na_2S_2O_3$ equals 1 mg O_2/ℓ as D.O.

E. CALCULATIONS

$$\text{D.O.} = \text{m}\ell \text{ of } Na_2S_2O_3 \text{ used} \times \frac{N}{0.025}$$

F. NOTE

The $Na_2S_2O_3$ should be standardized fairly frequently so that appropriate correction of measured D.O. for normality changes can be made.

BIOCHEMICAL OXYGEN DEMAND (BOD)

A. GENERAL

1. References: *Standard Methods* (1975) pp. 543-550.

2. Outline of Method: BOD determines the relative oxygen necessary for biological oxidation of wastewaters, effluents, and polluted waters. It is the only test available to determine the amount of oxygen required by bacteria while stabilizing decomposable organic matter. Complete stabilization requires too long an incubation period for practical purposes; therefore, the 5 day period has been accepted as a standard. Samples are incubated in the dark at 20 ± 1°C. Dissolved Oxygen levels are measured initially and at the end of the 5 day period using the Winkler with Azide Modification technique.

B. SPECIAL REAGENTS

A. For dilution water

1. Phosphate Buffer Solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 44.6 g $Na_2HPO_4 \cdot 12\ H_2O$, and 1.7 g NH_4Cl in about 500 mℓ of distilled water and dilute to 1 liter. (33.4 g $Na_2HPO_4 \cdot 7H_2O$).

2. Magnesium Sulfate Solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.

3. Calcium Chloride Solution: Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 liter. (36.4 g $CaCl_2 \cdot 2H_2O$).

4. Ferric Chloride Solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 liter.

B. For dechlorination

5. Sodium Sulfite Solution, 0.025 N: Dissolve 1.575 g anhydrous Na_2SO_3 *in* 1 liter distilled water. Prepare fresh when needed.

C. For pH adjustment

6. Acid and Alkali Solutions, 1 N: H_2SO_4, NaOH.

D. Dissolved oxygen (see DO section for method and reagents).

C. STANDARDIZATION

See Dissolved Oxygen.

D. PROCEDURE

1. Undiluted Samples: High quality water whose 5 day BOD is less than 7 mg O_2/ℓ. Initial DO should be near saturation levels; if not, aerate sample to saturation. Record initial DO and incubate at 20°C for five days repeating DO procedure.

$$\text{mg } BOD_5/\ell = D_1 - D_2$$

where

D_1 = initial dissolved oxygen
D_2 = 5 day dissolved oxygen

2. Diluted Samples: Because of the limited solubility of oxygen in water, samples with suspected high BOD's must be diluted.

Distilled water is aerated with a supply of clean compressed air. Dilution water should be at 20 ± 1°C. Add 1 mℓ each of phosphate buffer, magnesium calcium, and ferric solutions for each liter of dilution water needed. If dilution water is stored, add phosphate buffer just prior to use.

Prepare 2 sets of BOD bottles, including dilution water blanks; one set is for initial and the other set is for the 5 day oxygen determination. Fix one set and incubate the other at 20°C.

Make several dilutions of prepared sample so as to obtain sufficient oxygen depletions.

0.1-1.0%	for strong trade wastes
1-5%	for raw and settled sewage
5-25%	for oxidized effluents
25-100%	for polluted rivers

$$\text{mg } BOD_5/\ell = \frac{(D_1 - D_2) - (B_1 - B_2)}{P}$$

where

B_1 = initial DO of dilution water blank
B_2 = 5 day DO of dilution water blank
P = decimal fraction of sample used

3. Chlorinated Samples: Chlorinated samples are neutralized by sodium sulfite. The appropriate quantity of Na_2SO_3 solution

to add to the sample is determined on a 100-1000 mℓ portion of the sample by adding 10 mℓ of a 1:50 H_2SO_4 solution, followed by 10 mℓ of a KI solution (10 g in 100 mℓ) and titrating with 0.025 N Na_2SO_3 to the starch-iodide endpoint. Add to a volume of sample the quantity of Na_2SO_3 determined above. Test an aliquot of Na_2SO_3 treated sample by above method to check for residual chlorine. Use the treated sample for BOD test as in section 2.

4. Caustic Alkaline or Acidic Samples: Neutralize to about pH 7.0 with 1 N H_2SO_4 or NaOH using a pH meter.

5. Seeding: This procedure is used on neutralized chlorine residuals and other samples which need a biological population capable of oxidizing the organic matter in the wastewater. Standard seed material is settled raw domestic sewage which has been stored at 20°C for 24-36 hours. Use 2 mℓ per liter of diluted sample (0.2 percent). A seeded blank must be run with samples. A seed correction factor, f, is determined by setting up a separate series of seed dilutions and choosing the one dilution resulting in 40-70 percent oxygen depletion. The seed dilution giving 40-70 percent depletion is then designated as $B_1^{\dagger}$ and $B_5^{\dagger}$ for oxygen levels initially and after 5 days.

$$\text{mg } BOD_5/\ell = \frac{(D_1 - D_5)_{\text{sample}} - (B_1 - B_5)_{\text{seed blank}}\, f}{P}$$

where $f = \dfrac{\text{percent seed in sample - (should be 0.2 percent)}}{\text{percent seed with 40-70 percent oxygen depletion in series}}$

TABLE 24·1 BOD measurable with various dilutions of samples

Using per cent mixtures		*By direct pipetting into 300-ml bottles*	
% mixture	*Range of BOD*	*ml*	*Range of BOD*
0.01	20,000–70,000	0.02	30,000–105,000
0.02	10,000–35,000	0.05	12,000– 42,000
0.05	4,000–14,000	0.10	6,000– 21,000
0.1	2,000– 7,000	0.20	3,000– 10,500
0.2	1,000– 3,500	0.50	1,200– 4,200
0.5	400– 1,400	1.0	600– 2,100
1.0	200– 700	2.0	300– 1,050
2.0	100– 350	5.0	120– 420
5.0	40– 140	10.0	60– 210
10.0	20– 70	20.0	30– 105
20.0	10– 35	50.0	12– 42
50.0	4– 14	100	6– 21
100	0– 7	300	0– 7

CHEMICAL OXYGEN DEMAND

(Dichromate Reflux Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 550-554.

2. Outline of Method: The chemical oxygen demand (C.O.D.) determination provides a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. The dichromate reflux method has been selected for the C.O.D. determination because it has advantages over other oxidants in oxidizability, applicability to a wide variety of samples, and ease of manipulation. Most types of organic matter are destroyed by boiling a mixture of chromic and sulfuric acids. A sample is refluxed with known amounts of potassium dichromate and sulfuric acid, and the excess dichromate is titrated with ferrous ammonium sulfate. The amount of oxidizable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed. This technique is designed for typical sewage but there are many modifications possible for other samples; see *Standard Methods*, (1975) for details.

B. SPECIAL REAGENTS

1. Distilled Water: High quality, free of organic matter.

2. Mercuric Sulfate: Analytical grade, used to complex chlorides and remove them from the reaction.

3. Ferroin Indicator Solution: Dissolve 1.485 g 1, 10 phenanthroline monohydrate, together with .695 g $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mℓ.

4. Conc. Potassium Dichromate Solution, 0.25 N: Dry approx. 15 g of $K_2Cr_2O_7$ at 103°C for 2 hours. Dissolve exactly 12.259 g of the specially dried orange crystal in DW and dilute to exactly 1 liter. Nitrite-Nitrogen exerts a C.O.D. of 1.14 mg per mg NO_2-N. Add 0.12 g of Sulfamic Acid/liter of conc. dichromate solution will eliminate the interference of nitrites up to 6 mg/liter in the sample, if a 20 mℓ sample is used. (Note: Use Primary Standard Grade Dichromate.)

5. Conc. Sulfuric Acid Reagent: Add 22 g of Silver Sulfate (Ag_2SO_4) to a full 9 lbs. of conc. H_2SO_4. Allow 1-2 days for dissolution and then attach to the automatic filling acid burette. Silver sulfate catalyst used to more effectively oxidize straight chain organic compounds.

6. Ferrous Ammonium Sulfate Solution, approximately 0.25 N: Dissolve 98 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in approximately 600 mℓ DW. Add 20 mℓ of conc. H_2SO_4; cool; dilute to 1 liter with DW.

7. Dilute F.A.S. Solution, approximately 0.10 N: Dilute 400 mℓ of the 0.25 N F.A.S. solution to 1 liter with DW. *Standardize Daily.*

8. Chromic Acid Cleaning Solution: *Use Extreme Caution.* This solution may be re-used several times. *Slowly and Carefully* add 1 liter of conc. H_2SO_4 to 35 mℓ of saturated $Na_2Cr_2O_7$ solution. (p. 336 *S. M.*)

9. 50 Percent v/v H_2SO_4 Cleaning Solution: Add 500 mℓ conc. H_2SO_4 to 500 mℓ of DW. Cool, and dilute to 1 liter--used for glass bead cleaning.

10. Preparation of Glassware and Equipment: Care and cleaning of glassware is of *Extreme Importance* in the C.O.D. determination, since a very small amount of organic contamination will drastically change the results. Flasks and condensers should be rinsed carefully with DW prior to each use.

11. Glass Beads: Glass beads may be used repeatedly if they are soaked in the 50 percent H_2SO_4 Cleaning Solution; rinsed with DW, and air dried in the oven after each use. Cleaned beads should be stored in a covered bottle.

12. Chromic Acid Cleaning: When heavy deposits build up on the reflux flasks, they should be treated with chromic acid cleaning solution. Add about 100 mℓ of the cleaning solution to each flask; let stand 1 or 2 days. Pour the cleaning solution back into its bottle for re-use.

    Use Extreme Caution in Handling This Cleaning Solution. Wear Gloves.

    Wash and rinse the flasks with detergent and water; rinse with HCl dilute solution to remove traces of dichromate and rinse several times with DW.

C. STANDARDIZATION - Must Accompany Each Set of Determinations

1. Standardization of F.A.S. Titer: Dilute exactly 10 mℓ of 0.25 N Dichromate Solution to approximately 100 mℓ with DW. Add 30 mℓ of conc. H_2SO_4 Reagent, swirling while adding. Cool;

add 3-4 drops of Ferroin Indicator, and titrate to the red endpoint with Dilute F.A.S. Solution.

$$N = \text{F.A.S. Normality} = \frac{(m\ell\ K_2Cr_2O_7)\ (N\ K_2Cr_2O_7)}{m\ell\ \text{F.A.S. used}}$$

$$= (10)\ (0.25)/m\ell\ \text{F.A.S. used}$$

2. Distilled Water Blanks: Run 3 DW Blanks along with each set of samples tested. Adopting exactly the same procedure given for the samples, set up, reflux, and titrate 3 Blanks using 20 mℓ of quality DW instead of 20 mℓ of sample. The average volume of F.A.S. used in titrating these blanks will be "A" in the equation for calculating C.O.D. in mg/liter.

3. Standard Solution: Potassium acid phthalate has a theoretical C.O.D. of 1.176 g/g; therefore, dissolve .4251 g $KHC_8H_4O_4$ (also called potassium biphthalate) in distilled water and dilute to 1 liter for a 500 mg/ℓ C.O.D. solution. (A 98-100 percent recovery of the theoretical oxygen demand can be expected with potassium biphthalate.)

D. PROCEDURE

1. Place 4-5 cleaned, dried glass beads in each of the required 300 mℓ refluxing flasks. (One flask per sample and three flasks for the DW Blanks) DW rinsed flasks; set up F.A.S. standard now.

2. Add 1-2 scoops (approx. 0.4 g) of Mercuric Sulfate to each flask. Such an addition will complex any chloride ions present (up to 40 mg Cl^-) and prevent their interference. Add 20.0 mℓ sample, or an aliquot diluted to 20.0 mℓ with distilled water and mix.

3. Add exactly 10 mℓ of 0.25 N $K_2Cr_2O_7$; swirl and slowly add 30 mℓ of the conc. H_2SO_4 Reagent--swirling the solution throughout the acid addition. The reflux mixture must thoroughly mixed before the heat is applied. Acid volume: sample + dichromate volume ratio must be 1:1.

4. Attach condenser and turn on hot plate to the highest temperature. Be absolutely sure that the cold water is circulating through the water jackets surrounding the condensers before switching on the hot plate. Reflux for exactly 2 hours.

5. After 2 hour reflux period, turn off the heat and allow the samples to cool to room temperature, leaving the flasks and the condensers connected.

6. Wash down the condensers with 40 mℓ of DW; remove flasks from the condensers; dilute mixture with 50 mℓ distilled water, cool to room temperature. Add 3 drops of Ferroin Indicator and titrate with the Standard Dilute F.A.S. (approx. 0.10 N Solution). The endpoint is indicated by a blue-green to reddish-brown color change. Volume of F.A.S. used for the blanks will be "*A*" and the volume used for the sample will be "*B*" in the formula. Caution: Ferroin Indicator is destroyed when added to hot solution.

E. CALCULATIONS: COD formula

$$\text{mg/ℓ COD} = \frac{(A\text{-}B)\ N\ (8000)}{\text{mℓ sample}}$$

Where:

COD = chemical oxygen demand from dichromate
A = mℓ F.A.S. used for blank
B = mℓ F.A.S. used for sample
N = normality of F.A.S.

If 10 mℓ 0.25 N $K_2Cr_2O_7$ used for titer standardization and 20 mℓ sample volumes used:

$$\frac{N(8000)}{\text{mℓ sample}} = \frac{(\text{mℓ } K_2Cr_2O_7)\ (\text{N } K_2Cr_2O_7)\ (8000)}{(\text{mℓ sample})\ (\text{mℓ F.A.S. used to standardize})}$$

$$= \frac{(10)\ (0.25)\ (8000)}{(20)\ (\text{mℓ F.A.S. used to standardize})}$$

$$= \frac{(2.5)\ (400)}{(\text{mℓ F.A.S. used to standardize})}$$

$$= \frac{1000}{\text{mℓ F.A.S. used to standardize}}$$

Therefore:

$$\text{mg/ℓ COD} = \frac{1000}{\text{mℓ F.A.S. used to standardize}}\ (A\text{-}B)$$

F. NOTES

For low level COD use diluted solutions of both $K_2Cr_2O_7$ and F.A.S. For example 0.025 $K_2Cr_2O_7$ titrated with 0.01 N F.A.S.

LOW-LEVEL FLUORIDE

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 391-393.

2. Outline of Method: The fluoride electrode is a selective ion sensor. The fluoride ion-selective electrode can be used to measure the activity or the concentration of fluoride in aqueous samples by use of an appropriate calibration curve. However, the fluoride activity depends on the total ionic strength of the sample. The electrode does not respond to bound or complexed fluoride. A buffer solution of high ionic strength may be added to swamp variations in sample ionic strength.

B. SPECIAL REAGENTS AND EQUIPMENT

1. Expanded scale or digital pH meter or ion-selective meter.

2. Sleeve-type reference electrode - Orion #90-01-00, Reckman #40463 or Corning #476012.

3. Fluoride electrode - Orion #94-09.

4. Magnetic stirrer.

5. Total ionic strength adjustment buffer (TISAB). Place approximately 500 mℓ distilled water in a 1 liter beaker and add 57 mℓ glacial acetic acid, 58 g NaCl, and 4.0 g 1, 2 cyclohexylenediamine tetraacetic acid (CDTA).* Stir to dissolve. Place beaker in cool water bath for cooling. Immerse a calibrated pH electrode into the solution and slowly add approximately 5 M NaOH until the pH is between 5.0 and 5.5. Cool to room temperature and dilute to 1 liter.

* Low level samples containing less than 0.4 mg/ℓ fluoride and containing no complexing agents such as aluminium or iron may be run using TISAB without CDTA.

C. STANDARDIZATION

1. Stock fluoride solution: Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1 liter.

   1.00 mℓ = 100 μg F^-

2. Prepare a dilute standard solution by adding 10.0 mℓ of stock fluoride solution to distilled water and dilute to

100 mℓ.

1.00 mℓ = 10 μg F^-

3. Calibration curve for low-level measurements:

   a. To a 150 mℓ plastic beaker add 50 mℓ TISAB and 50 mℓ distilled water. Set the function switch to REL MV. Place electrodes in solution and stir thoroughly.

   b. Add increments of the 10 mg/ℓ F^- standard using the following steps. Measure the electrode potential after each increment.

Step	Added Volume	Concentration (mg/ℓ)
1	0.1 mℓ	0.01
2	0.1 mℓ	0.02
3	0.2 mℓ	0.04
4	0.2 mℓ	0.06
5	0.4 mℓ	0.10
6	2.0 mℓ	0.29
7	2.0 mℓ	0.48
8	2.0 mℓ	0.65
9	2.0 mℓ	0.83
10	2.0 mℓ	0.99
11	2.0 mℓ	1.15
12	2.0 mℓ	1.30

D. PROCEDURE

1. Add 50 mℓ TISAB to 50 mℓ sample.

2. Rinse electrodes, blot dry, and place in sample. Stir thoroughly.

3. Record the electrode potential for the sample.

E. CALCULATIONS

1. Using semi-logarithmic graph paper plot the standard concentrations (log axis) against the potential (linear axis) to determine the calibration curve.

2. Determine the concentration of the sample from the calibration curve.

F. NOTES

Samples and standards should always be stored in plastic containers as fluoride reacts with glass.

SOLUBLE IRON

A. GENERAL

1. Reference: Strickland and Parsons, *A Practical Handbook of Seawater Analysis* (1968) pp. 99-100 (Particulate), pp. 105 - 107 (Soluble).

2. Outline of Method: The filtered water sample is treated with hydrochloric acid and then reacted with the bathophenanthroline solution in an acetate buffer in the presence of hydroxylamine. The absorbance if the colored complex thus formed is measured at 533 mμ and 650 mμ.

B. SPECIAL REAGENTS

1. Bathophenanthroline Solution: Dissolve 0.070 g of 4, 7-diphenyl-1, 10-phenanthroline (bathophenanthroline) in 100 mℓ of ethyl alcohol and then add 100 mℓ of distilled water. Keep in a well stoppered polyethylene bottle; stable indefinitely.

2. Iso-amyl Alcohol: Reagent grade.

3. Hydroxylamine Hydrochloride: Dissolve 20 g hyrdoxylamine hydrochloride ($NH_2OH \cdot HCl$) in 200 mℓ of DW. **Place this solution in a separatory funnel; add 10 mℓ of the Batho-solution, and 20 mℓ of iso-amyl alcohol. Extract any iron dissolved in the reagent by shaking vigorously for 1 minute; allowing the layers to separate, and drawing off the lower level into another separatory funnel. Repeat from (**) above until the extracts are colorless. Allow the final extract to separate for 5-10 minutes before running the hydroxylamine solution into a well stoppered 250 mℓ polyethylene bottle.

4. Sodium Acetate Buffer: Dissolve 75 g of sodium acetate tri-hydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in 100 mℓ of DW (this will make approx. 150 mℓ of solution). Place this solution into a separatory funnel; add 2 mℓ of the hydroxylamine solution and 5 mℓ of the batho-solution; allow the mixture to stand for 15 minutes. Extract the solution with 10 mℓ portions of iso-amyl alcohol until the alcohol layers are colorless. Add 5 mℓ of the batho-solution and extract once again for confirmation that all iron has been removed. Allow final extract to separate for 10-15 min. and store the aqueous solution in a well stoppered polyethylene bottle.

5. Iron Extraction Reagent: Add 20 mℓ of conc. HCl to DW and dilute to 500 mℓ with DW.

C. STANDARDIZATION

1. Stock Conc. Ferrous Ammonium Sulfate (FAS) Standard: Dissolve exactly 3.510 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 400 mℓ DW in which is 10 mℓ conc. H_2SO_4 and dilute to exactly 500 mℓ with DW.

   This salt should not be oven dried since it is in an hydrated form. Iron is very unstable in solution and must be freshly prepared at each standardization.

   1 mℓ = 1 mg Fe

2. One could use the standardized FAS from the COD test as a source of known standard.

3. Standard Curve:

   0 µg Fe/ℓ to 320 µg Fe/ℓ

D. PROCEDURE

1. Because iron settles out of the samples and is absorbed into the walls of the container quite readily, analysis of the samples should commence without delay in order to obtain the most precise data. Note that particulate iron involves same technique yet run on an unfiltered sample.

2. Measure 50 mℓ of the filtered sample (GF/C) into a 150 mℓ beaker. Add 5 mℓ of the extraction reagent and 1 mℓ of hydroxylamine hydrochloride solution; mix.

3. After 5 minutes, add 1 mℓ of sodium acetate solution; 2.5 mℓ of batho-solution; mix, and allow the solution to stand for 10 minutes.

4. Record the absorbance of the sample against DW in a 5 cm cell at 533 mµ and also at 650 mµ. The measurement taken at 650 mµ is used as a correction factor for turbidity of the sample.

E. CALCULATIONS

Calculate the concentration of soluble iron by subtracting the absorbance at 650 mµ from the absorbance at 533 mµ, subtract reagent blank to give corrected optical density; obtain the result from the slope-intercept formula of the standard curve.

TOTAL IRON

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 207-213.

2. Outline of Method: Iron is brought into solution, is reduced to the ferrous state by boiling with acid and hydroxylamine and treated with 1, 10-phenanthroline at pH 3.2 - 3.3. Three molecules of phenanthroline chelate each atom of ferrous iron to form orange-red complex. The colored solution obeys Beer's law.

B. SPECIAL REAGENTS

1. Hydrochloric Acid, Concentrated

2. Hydroxylamine Solution: Dissolve 10 g $NH_2OH \cdot HCl$ in 100 mℓ distilled water.

3. Ammonium Acetate Buffer Solution: Dissolve 250 g ammonium acetate ($NH_4C_2H_3O_2$) in 150 mℓ distilled water. Add 700 mℓ concentrate (glacial) acetate acid to form slightly more than 1 liter of solution.

4. Phenanthroline Solution: Dissolve 100 mg 1, 10-phenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$) in 100 mℓ distilled water by stirring and heating to 80°; do not boil. Discard the solution if it darkens. Heating is not necessary if 2 drops of concentrate HCl are added to the distilled water. (Note that 1 mℓ of this reagent is sufficient for no more than 100 mg Fe.)

C. STANDARDIZATION

1. Stock Conc. Ferrous Ammonium Sulfate (FAS) Standard: Dissolve exactly 3.510 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 400 mℓ DW in which is 10 mℓ conc. H_2SO_4 and dilute to exactly 500 mℓ with DW. This salt should not be oven dried since it is in a hydrated form. Iron is very unstable in solution and must be freshly prepared at each standardization.

$$1 \text{ mℓ} = 1 \text{ mg Fe}$$

2. One could use the standardized FAS from the COD test as a source of known standard.

3. Standard Curve:

20 µg Fe/ℓ to 4000 µg Fe/ℓ

D. PROCEDURE

1. Because iron settles out of the samples and is absorbed into the walls of the container quite readily, analysis of the samples should commence without delay in order to obtain the most precise data. Note that soluble iron involves same technique but is run on a filtered sample.

2. Measure 50 mℓ of sample into a 125 mℓ Erlenmeyer flask. (If the sample contains more than 2 mg/ℓ Fe, dilute and accurately measure aliquot containing not more than 100 mg per 50 mℓ.)

3. Add 2 mℓ concentrate HCl and 1 mℓ hydroxylamine solution.

4. Add a few glass beads and heat to boiling to insure dissolution of all the iron, continue boiling until the volume is reduced to 15-20 mℓ.

5. Cool to room temperature and transfer to a 50 or 100 mℓ volumetric flask. Add 10 mℓ ammonium acetate buffer solution and 2 mℓ phenanthroline solution, and dilute to the mark with distilled water.

6. Mix thoroughly and allow at least 10-15 minutes for maximum color development.

7. Record the absorbance of the sample against DW in a 5 cm cell at 533 mµ and also at 650 mµ. The measurement taken at 650 mµ is used as a correction factor for turbidity of the sample.

E. CALCULATIONS

Calculate the concentration of soluble iron by subtracting the absorbance at 650 mµ from the absorbance at 533 mµ, subtract reagent blank to give corrected optical density; obtain the result from the slope-intercept formula of the standard curve.

REACTIVE NITRITE

(Diazotization Method)

A. GENERAL

1. Reference: Strickland and Parsons, *A Practical Handbook of Seawater Analysis* (1968) pp. 77-80 and *Standard Methods* (1975) pp. 434-436.

2. Outline of Method: The nitrite is allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with the naphthylethylenediamine reagent and forms a highly colored azo dye, the absorbance of which is measured spectrophotometrically.

B. SPECIAL REAGENTS

1. Sulphanilamide Solution: Dissolve 5 g of sulphanilimide *in* a mixture of 50 mℓ of conc. hydrochloric acid (sp. gr. 1.18) and about 300 mℓ of distilled water. Dilute to 500 mℓ with water. Stable many months.

2. N-(1-Naphthyl)-Ethylenediamine Dihydrochloride Solution: Dissolve 0.50 g of the reagent powder *in* 500 mℓ of distilled water and store in a dark bottle. Stable 1 month.

C. STANDARDIZATION

1. Standard Nitrite Solution: A small amount of anhydrous analytical reagent quality sodium nitrite ($NaNO_2$) should be dried at 110 C for 1 hour. Dissolve 0.345 g of the dried $NaNO_2$ in distilled water and dilute to 1 liter. Store in dark bottle with 1 mℓ of chloroform as a preservative. Stable 1-2 months.

$$1\ m\ell \equiv 70\ \mu g\ NO_2\text{-}N$$

2. Standard Curve:

$$0 \text{ to } 140\ \mu g\ NO_2\text{-}N/\ell$$

3. Procedure for daily standardization:

	Concentration
A. Dilute 1 mℓ 70 µg/mℓ → 100 mℓ	70 µg/100 mℓ 700 µg/ℓ .7 µg/mℓ
B. Dilute 5 mℓ 0.7 µg/mℓ → 100 mℓ	3.5 µg/100 mℓ 35 µg/ℓ

This 35 µg NO_2-N/ℓ Standard can then be used on either the 1 or 5 cm pathlengths.

D. PROCEDURE

1. Analysis to be performed upon GF/C filtered samples. If apparent coloration remains in sample, run turbidity correction test. To a 30 mℓ aliquot of sample add 1 mℓ of sulphanilamide and measure absorbance.

2. Measure a 50 mℓ sample or an aliquot diluted to 50 mℓ into a 125 mℓ Erlenmeyer flask; samples 15-25 C.

3. Add 1 mℓ of sulphanilamide sol.; *mix*, and allow the reagent to react for 2-8 min.

4. Add 1 mℓ of the naphthylethylenediamine solution. Ten minutes is required for complete color development, with the color being stable for 2 hrs. Samples follow Beer's Law and may be diluted 1:2 after the addition of reagents.

5. Measure absorbances of the solutions following the 10 min. reaction period in 1 or 5-cm cells, against distilled water at 543 mµ. Subtract off turbidity absorbance to give corrected optical density.

6. Calculate NO_2-N conc. using the slope-intercept formula from the standard curve.

E. CALCULATION

$$m^{-1} = \frac{\Delta \text{ concentration}}{\Delta \text{ absorbance}} = \frac{35 - 0}{Abs_{std} - Abs_{blank}}$$

d.f. = dilution factor

Sample Concentration µg NO_2-N/ℓ = $(Abs_{sample} - Abs_{blank})(m^{-1})(d.f.)$

If a 10 percent sample is used: d.f. = 10, if a 5 percent sample is used: d.f. = 20.

REACTIVE NITRATE

(Cadmium-Reduction Method)

A. GENERAL

1. Reference: Strickland and Parsons, *A Practical Handbook of Seawater Analysis* (1968) pp. 71-76.
   Standard Methods (1975) pp. 423-427.

2. Outline of Method: The nitrate in the sample is reduced almost quantitatively to nitrite when it is run through a column containing cadmium filings loosely coated with metallic copper. The nitrite thus produced is measured by the method outlined for Reactive Nitrite. A correction may be made for any nitrite initially present in the sample by subtracting μg NO_2-N/ℓ from μg (NO_3-N + NO_2-N)/ℓ.

B. SPECIAL REAGENTS

1. Conc. Ammonium Chloride Solution: Dissolve 175 g of analytical quality NH_4Cl *in* 500 mℓ of DW and store in glass or plastic bottle.

2. Dilute Ammonium Chloride Solution: Dilute 50 mℓ of conc. NH_4Cl solution to 2000 mℓ with DW. Store in glass or plastic.

3. Methylene Chloride or Methylenedichloride.

4. 5 Percent v/v Solution of HCl: Add 25 mℓ of conc. HCl to 300 mℓ DW and dilute to 500 mℓ.

5. 2 Percent Copper Sulfate: Dilute 10 g solid $CuSO_4 \cdot 5H_2O$ to 500 mℓ with DW. (Copper Sulfate Pentahydrate)

6. Sulphanilamide Solution:
   N-(1-Naphthyl)-Ethylenediamine Dihydrochloride Solution:
   See Nitrite.

Preparation of Reducing Columns
Cadmium (Copper Plate) Filings

0.5 mm < size < 2 mm ~ 50 g/column

1. Weigh 50 g of dried chips of cadmium for each column, or empty used cadmium from old columns. Wash cadmium chips twice with methylenedichloride and air dry to remove grease.

2. Wash chips briefly with about 400 mℓ of 5 percent v/v HCl and then rinse 2 or 3 times with DW. Supernatant should no longer be acid (pH greater than 5).

3. Stir 100 g filings with 500 mℓ of 2 percent w/v $CuSO_4 \cdot 5H_2O$ (1.28 percent w/v $CuSO_4$) until all blue color has left and semicolloidal copper particles begin to enter the supernatant liquid.

4. Plug bottom of reduction column with very fine copper turnings (glass wool).

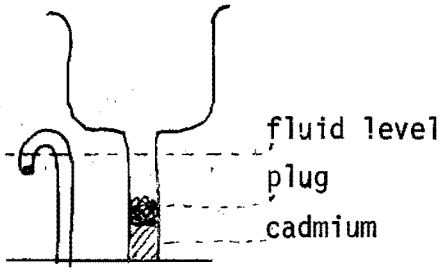
5. Fill column with supernatant liquor from preparation of cadmium.

6. Pour in cadmium filings (30 cm length), tap to insure filings are <u>well</u> settled.

7. Wash column with dilute NH_4Cl.

8. Flow rate $\frac{100\ m\ell}{8\ min}$ to $\frac{100\ m\ell}{12\ min}$.

9. Plug top of column with copper "wool." Prime each column by running 500 mℓ of 100 µg/ℓ standard solution.



10. When column not in use, cadmium must be completely covered by dilute NH_4Cl. Column must be covered with aluminum foil.

11. When standards show that the efficiency of columns is low, the filings need to be retreated:

    a. Empty four columns into beaker and stir vigorously with 300 mℓ of 5 percent v/v HCl solution.

    b. Decant acid and repeat.

    c. Wash metal with 200-300 mℓ portions of distilled water until supernatant is no longer acid (pH ≥ 5), decant liquid to leave metal as dry as possible.

    d. Retreat metal with $CuSO_4$ solution (see 3 above). Regenerated cadmium should be enough for 3 columns.

C. STANDARDIZATION

1. Stock Standard Conc. Potassium Nitrate: Dry approximately 5 g of anhydrous KNO_3 in the oven at 100°C for 1 hr. Dissolve exactly 3.61 g of KNO_3 in DW and dilute to exactly 500 mℓ with DW. Stable for 6 months.

   1 mℓ = 1 mg NO_3-N

2. Standard Dilute Potassium Nitrate: Dilute 10 mℓ of conc. stock standard solution to 1000 mℓ with DW; make fresh daily.

   1 mℓ = 10 µg NO_3-N

3. Measure 10 mℓ of the dilute standard nitrate solution and dilute to exactly 100 mℓ with DW.

   1 mℓ = 1.0 µg NO_3-N

4. Standard Curve:

   0 to 150 µg N/ℓ

5. Procedure for daily standardization.

	Concentration
A. Dilute 1 mℓ 1 mg/mℓ → 100 mℓ	1 mg/100 mℓ 10 mg/ℓ
B. Dilute 1 mℓ 10 µg/mℓ → 100 mℓ	.01 mg/mℓ or 10 µg/mℓ 10 µg/100 mℓ 100 µg/ℓ

D. PROCEDURE

1. Calibrate the rate of flow in the reducing columns with dilute NH_4Cl so that 10 mℓ of solution passes through the column in 1 min. Allow the dilute NH_4Cl used in the calibration to drain out.

2. Measure 100 mℓ of GF/C filtered sample into a 250 mℓ beaker. The concentration of the samples should not be higher than 200 µg NO_3-N/liter. Samples known to exceed this concentration should be diluted prior to reduction. Add 2 mℓ of conc. NH_4Cl to each of the 100 mℓ samples and mix. The slight acidification of the sample by the addition of NH_4Cl greatly slows the deactivation process; column good for at least 100 samples.

3. Pour in 5 mℓ sample and allow it to pass through, then add the remaining sample into the rinsed, drained columns and collect the first 40 mℓ of sample in a 50 mℓ graduated cylinder.

Discard this portion of the sample and collect the remaining portion in the 250 mℓ beaker. The passage of the 40 mℓ flushes the column of the preceding sample.

4. As soon as the sample has passed through the columns, measure a 50 mℓ aliquot in the 50 mℓ graduate, discard the remaining portion and return the 50 mℓ aliquot to the beaker.

5. If the above procedure is followed, it is not necessary to rinse either the graduate or the columns between samples unless extreme changes from high to low concentrations are encountered.

6. Following the method for NO_2-N determination, obtain the absorbance of the reduced sample at 543 mμ in a 1 or 5 cm cell.

7. Calculate the concentration of NO_3-N + NO_2-N by plugging the absorbance of the sample into the slope-intercept formula for the NO_3-N standard curve. Subtract as follows the NO_2-N value from this total figure to determine the amount of NO_3-N present:

$$NO_3^-\text{-N} = \Sigma(NO_3^- + NO_2^-)N - 0.95\ (NO_2^-\text{-N}).$$

Note: Samples follow Beer's Law and may be diluted after the reagents have been added up to a 1:2 dilution. Samples which require more than a 1:2 dilution should be diluted *before reduction*.

E. CALCULATION

$$m^{-1} = \frac{\Delta \text{ concentration}}{\Delta \text{ absorbance}} = \frac{100 - 0}{Abs_{std} - Abs_{blank}} \qquad \text{d.f.} = \text{dilution factor}$$

$$\text{Sample Concentration } \mu g(NO_3 + NO_2)\text{-N}/\ell = (Abs_{sample} - Abs_{blank})(m^{-1})(\text{d.f.})$$

If a 10 percent sample is used: d.f. = 10
If a 5 percent sample is used: d.f. = 20.

AMMONIA

(Method 1: Indophenol)

A. GENERAL

1. Reference: Solorzano, Lucia, *Limnology and Oceanography*, Vol. 14, No. 5, September 1969, pp. 799-801 and *Standard Methods* (1975) pp. 416-417.

2. Outline of Method: Blue color of indophenol obtained by the reaction at high pH of ammonia, phenol, and hypochlorite is being measured. This method eliminates interference due to precipitation by complexing Mg and Ca with citrate.

B. SPECIAL REAGENTS

1. Ammonia free distilled water: A. F. D. W.

2. Phenol-alcohol solution: 10 g of phenol *in* 100 mℓ of 95 percent ethyl alcohol.

3. Sodium nitroprusside: 0.5 g *in* 100 mℓ of A.F.D.W. in amber bottle.

4. Alkaline solution: 100 g of trisodium citrate and 5 g of NaOH *in* 500 mℓ A.F.D.W.

5. Sodium hypochlorite solution: commercial product Clorox.

6. Oxidizing solution: 100 mℓ of the alkaline solution and 25 mℓ of the hypochlorite solution mixed together and used the same day for analysis.

C. STANDARDIZATION

1. Stock Standard Conc. Ammonium Chloride Solution: *Dissolve 1.91 g of reagent quality NH_4Cl in 490 mℓ A.F.D.W. and dilute to 500 mℓ.

$$1\ m\ell \equiv 1\ mg\ NH_3\text{-}N$$

*NOTE:
Dry 2-3 g of the NH_4Cl at 100 C for 1 hour.

2. Standard Curve

Range of ammonia concentrations is 10 µg/ℓ - 200 µg/ℓ. Below 10 µg/ℓ the absorbance is not appreciably different from the

blank value. The analysis is more reliable at higher concentrations within the range.

3. Procedure for daily standardization

	Concentration
A. Dilute 1 mℓ 1 mg/mℓ → 100 mℓ	1 mg/100 mℓ 10 mg/ℓ
B. Dilute 1 mℓ 10 µg/mℓ → 100 mℓ	.01 mg/mℓ or 10 µg/mℓ 10 µg/100 mℓ 100 µg/ℓ

D. PROCEDURE

Sample concentrations must range from 10 µg/ℓ - 200 µg/ℓ. If the sample has a higher concentration, it must be diluted to within this range.

1. Consecutively add to a 50 mℓ filtered sample (or an aliquot diluted to 50 mℓ): 2 mℓ of phenol solution, *mix*, 2 mℓ nitroprusside solution, *mix*, and 5 mℓ of the oxidizing solution and *mix*.

2. Stopper flasks and allow color to develop for 2 hours in the dark; color good for 24 hours.

3. Read and record absorbance at 640 mµ.

E. CALCULATION

$$m^{-1} = \frac{\Delta \text{ concentration}}{\Delta \text{ absorbance}} = \frac{100 - 0}{Abs_{std} - Abs_{blank}} \qquad d.f. = \text{dilution factor}$$

$$\text{Sample Concentration, µg } NH_3\text{-N}/\ell = (Abs_{sample} - Abs_{\substack{weighted \\ blank}})(m^{-1})(d.f.)$$

If a 10 percent sample is used: d.f. = 10, if a 5 percent sample is used: d.f. = 20

Weighted Blank necessary because NH_3-N in blank (sometimes high) cannot always be found in sample tested.

Percent Sample	Weighted Blank Formula
100.	(.85)(.009) + (.15)(Blank) = .008 + (.15)(Blank)
50.	(.42)(.009) + (.58)(Blank) = .004 + (.58)(Blank)
20.	(.17)(.009) + (.83)(Blank) = .002 + (.83)(Blank)
10.	(.08)(.009) + (.92)(Blank) = .001 + (.92)(Blank)
<10.	Blank

AMMONIA NITROGEN

(Method 2: Distillation)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 412-415.

2. Outline of Method: Free ammonia nitrogen is quantitatively recovered by distillation of a sample near pH 7.4, a level which is maintained by adding a phosphate buffer system. The distillate is then tested for ammonia:

Range, mg/ℓ	Technique
.010-.500	Indophenol
.500-5.00	Nesslerization
> 5	Titration

NOTES

1. Dechlorination

2. Indophenol, distill if Alkalinity > 500 mg/ℓ or if sample turbid. Distill into H_2SO_4 absorbent.

3. pH adjustment critical at Ca^{++} > 250 mg/ℓ.

B. SPECIAL REAGENTS

1. Ammonia Free Distilled Water (A.F.D.W.): Add 0.1 mℓ conc. H_2SO_4 per liter of DW and redistill. For best results, make fresh prior to each test.

2. Phosphate Buffer, pH 7.4: Dissolve 14.3 g KH_2PO_4 and 68.8 g KH_2PO_4 in A.F.D.W. and dilute to 1ℓ.

3. Dechlorinating Agent: ($Na_2S_2O_3$)
Neutralizing Agent: ($NaOH$, H_2SO_4:1N) See *Standard Methods*.

4. Absorbent Solution: H_3BO_3, 2 percent: Dissolve 20 g H_3BO_3 in A.F.D.W. and dilute to 1ℓ. Each mg NH_3-N requires an additional 50 mℓ of boric acid solution for effective NH_3-N absorption.

5. Mixed Indicator: Dissolve 200 mg methyl red indicator in 100 mℓ 95 percent EtOH. Dissolve 100 mg methylene blue in 50 mℓ 95 percent EtOH. Combine the 2 solutions. Prepare monthly.

6. Absorbent Solution: Indicating H_3BO_3: Dissolve 20 g H_3BO_3 in A.F.D.W., add 10 mℓ mixed indicator solution and dilute to 1 ℓ. Prepare monthly. To be used for titration.

7. Absorbent Solution: H_2SO_4, 0.02N: See Alkalinity. Use this absorbent for indophenol technique.

8. Standard H_2SO_4, 0.02N Titer: See Alkalinity.

NOTE: To standardize this titer, use the Na_2CO_3 standard which has been incorporated into the indicating boric acid solution:

$$1.0\ m\ell\ H_2SO_4(0.0200N) \equiv 280\ \mu gN$$

9. Nessler Reagent: Dissolve 100 g HgI_2, and 70 g KI, in a small quantity of A.F.D.W., and add to this mixture slowly, with stirring, to a cool solution of 160 g NaOH in 500 mℓ A.F.D.W. Dilute to 1 ℓ. Store in rubber stoppered pyrex glassware and store out of direct sunlight.

   a. Develops characteristics within 10 min.
   b. Doesn't produce a precipitate within 2 hrs.

CAUTION: TOXIC

10. Dechlorinating Agent N/70: $Na_2S_2O_3$

11. Neutralizing Agent: 1 N NaOH
    1 N H_2SO_4

C. STANDARDIZATION

1. Stock Standard Conc. Ammonium Chloride Solution: Dry 2-3 g of reagent quality NH_4Cl at 100 C for 1 hr. Dissolve exactly 1.91 g of the dry NH_4Cl in 490 mℓ of A.F.D.W. and dilute to exactly 500 mℓ with A.F.D.W. Stable 6 months.

$$1\ m\ell = 1\ mg\ NH_3\text{-}N$$

2. Stock Standard Dilute NH_4-Cl Solution: Dilute 1 mℓ of the conc. solution to 100 mℓ with A.F.D.W.; make fresh daily.

$$1\ m\ell = 10\ \mu g\ NH_3\text{-}N$$

D. PROCEDURE

1. Place a 500 mℓ sample (or an aliquot diluted to 500 mℓ with A.F.D.W.) in a Kjeldahl flask.

   a. Dechlorinate and/or neutralize if necessary

NOTE: If NH_3-N < 100 µg/ℓ, concentrate a volume of 1000 mℓ to 500 mℓ.

2. Add 10 mℓ phosphate buffer.

NOTE: Samples > 250 mg Ca^{++}, add additional 10 mℓ buffer for each 250 mg Ca^{++} in sample.

3. Distill sample immediately after steaming out distillation apparatus using 500 mℓ A.F.D.W. plus 10 mℓ buffer.

4. Collect sample distillate with tip of delivery tube submerged in 50 mℓ of absorbent in a 500 mℓ flask.

   a. If H_3BO_3 is used, add additional 50 mℓ increments of H_3BO_3 for each mg NH_3-N distilled.

   b. Absorbent

      i. Indophenol: 0.02N H_2SO_4
      ii. Nesslerization: 2 percent H_3BO_3
      iii. Titration: 2 percent Indicating H_3BO_3

5. Collect 300 mℓ distillate. Lower delivery flask (free of contact with delivery tube) during the last minute or two of distillation and allow condenser and delivery tube to be cleansed.

6. Continue distillation so as to concentrate residue in Kjeldahl flask to 30-50 mℓ--throw excess distillate away. Save residue for organic nitrogen determination.

7. Test for NH_3-N in distillate by either titration, nesslerization or indophenol techniques.

F. NOTES

1. Preparation of glassware and distilling apparatus is of EXTREME IMPORTANCE for maintaining a high level of accuracy. Glassware used in this test should be set aside and marked for LOW LEVEL AMMONIA ANALYSIS ONLY. After washing glassware, the distillation apparatus should be steamed.

2. Nesslerization

   a. Measure a 50 mℓ aliquot of the distillate into a clean beaker which has been carefully rinsed with A.F.D.W.

   b. Add exactly 2 mℓ of Nessler reagent to the sample with a safety pipetting bulb. Draw the Nesslers from near the surface using extreme caution not to disturb the precipitate which settles to the bottom of the reagent bottle.

   c. Mix the Nesslerized sample immediately; wait exactly 15 min; mix again and read the absorbance at 410 mμ.

3. Acid titration

Titrate distillate with standard 0.02N H_2SO_4 titrant until indicator turns a pale lavender.

$$\text{mg N}/\ell = \frac{(D-E) \times 280}{\text{m sample}}$$

D: mℓ H_2SO_4 for sample
E: mℓ H_2SO_4 for blank

TKN
ORGANIC NITROGEN

(Digestion, Distillation)

A. GENERAL

1. Reference: *Methods for Chemical Analysis of Water and Wastes*, (EPA), 1976.

2. Outline of Method: In the presence of a strong acid, organically bound nitrogen is quantitatively converted to ammonia, which is distilled (to remove impurities) and collected in boric acid.

B. SPECIAL REAGENTS

1. Distilled water should be free of ammonia. Such water is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

   NOTE: All solutions must be made with ammonia-free water.

2. Mercuric Sulfate Solution: Dissolve 8 g red, mercuric oxide (HgO) in 50 mℓ of 1:5 sulfuric acid (10.0 mℓ conc. H_2SO_4: 40 mℓ distilled water) and dilute to 100 mℓ with distilled water.

3. Sulfuric Acid-Mercuric Sulfate-Potassium Sulfate Solution (Digestion Mixture): Dissolve 267 g K_2SO_4 in 1300 mℓ distilled water and 400 mℓ conc. H_2SO_4. While the solution is still hot, add 50 mℓ mercuric sulfate solution and dilute to 2 liters with distilled water.

4. Sodium Hydroxide-Sodium Thiosulfate Solution: Dissolve 500 g NaOH and 25 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 liter.

5. Boric Acid Solution: Dissolve 20 g boric acid, H_3BO_3, in water and dilute to 1 liter with distilled water.

6. Nessler Reagent: Dissolve 100 g of mercuric iodide and 70 g potassium iodide in a small volume of distilled water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 mℓ of distilled water. Dilute the mixture to 1 liter. The solution is stable for at least one year if stored in a pyrex bottle out of direct sunlight.

C. STANDARDIZATION

Standards are run at a concentration of 1 mg/ℓ. There is no distillation and an undistilled blank must be run as well as a distilled blank.

1. Stock Standard Conc. Ammonium Chloride Solution:* Dissolve 1.91 g of reagent quality NH_4Cl in 490 mℓ A.F.D.W. and dilute to 500 mℓ.

$$1\ m\ell \equiv 1\ mg\ NH_3\text{-}N$$

2. Standard Curve

		Concentration
a. Dilute 1 mℓ 1 mg/mℓ	→ 10 mℓ	10 mg/100 mℓ 100 mg/ℓ .1 mg/mℓ or 100 µg/mℓ
b. Dilute 1 mℓ 100 µg/mℓ	→ 100 mℓ	100 µg/100 mℓ 1000 µg/ℓ or 1 mg/ℓ

*Dry 2-3 g NH_4Cl at 100^0 for 1 hr.

D. PROCEDURE

1. Place 50 mℓ of total (unfiltered) sample into a 100 mℓ microkjeldahl flask. Add 10 mℓ of digestion mixture and 3-4 boiling chips (Tamer Tabs).

2. Place flask on the microkjeldahl apparatus (in a hood) and evaporate the mixture at low heat until ∿1/2 the sample is remaining. Increase heat, and evaporate until SO_3 fumes are given off and the solution turns colorless or *pale yellow*. Continue heating for 30 additional minutes. Cool the residue.

3. Transfer residue (using 500 mℓ of distilled water) into an 800 mℓ Kjeldahl flask.

4. Make the digestate alkaline by careful addition of 10 mℓ of sodium hydroxide-thiosulfate solution without mixing.

   NOTE: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause heavier solution to underlay the aqueous sulfuric acid solution without loss of free-ammonia. Do not mix until the digestion flask has been connected to the distillation apparatus. Connect the Kjeldahl flask to the condenser with the tip of condenser (or an extension of the condenser tip) below the level of the boric acid solution in the receiving flask.

   Distill 300 mℓ at the rate of 6-10 mℓ/min., into 50 mℓ of

2 percent boric acid contained in a 500 mℓ Erlenmeyer flask. These flasks should be marked at the 350 mℓ level.

5. When the 300 mℓ level has been reached, drop receiving flasks and allow the last 50 mℓ to drip into the flask.

6. Remove flask at the 350 mℓ level.

7. Measure 50 mℓ of distillate and add 2 mℓ of Nessler's Reagent. Read after exactly 20 minutes. (This exact timing necessitates addition of Nessler's to sample at some interval (1 minute) so as to allow spectrophotometric analysis at the correct time.)

8. Read samples utilizing a 1 cm path length at 425 mμ.

E. CALCULATION

$$m^{-1} = \frac{\Delta \text{ conc.}}{\Delta \text{ Abs}} = \frac{1 \text{ mg/ } -0}{\text{Abs}_{std} - \text{Abs}_{ub*}}$$

*ub = undistilled blank

$$\text{Sample concentration, mg NH -N/ℓ} = (\text{Abs}_{sample} - \text{Abs}_{db*})(m^{-1})(\text{d.f.})$$

*db = distilled blank

d.f. is generally 7 because the initial sample volume of 50 mℓ is diulted to 350 during the distillation.

F. NOTES

1. The distillation apparatus should be pre-steamed before use by distilling a 1:1 mixture of distilled water and sodium hydroxide-sodium thiosulfate solution until the distillate is ammonia-free. This operation should be repeated each time the apparatus is out of service long enough to accumulate ammonia (usually 4 hours or more).

2. Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

3. Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

OIL AND GREASE

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 513-516; and *Methods for Analysis of Organic Substances in Water*, USGS (1972).

2. Outline of Method: Liquid-liquid extraction is the method most widely employed in separating organic compounds from aqueous mixtures in which they are found or produced. The procedure involves the distribution of a solute between two immiscible solvents. The oils or greases are extracted from the aqueous solution by direct contact with various immiscible organic solvents. The organic solvent is then separated from the aqueous phase, dried and evaporated to determine the extractable residue by gravimetric techniques.

B. SPECIAL REAGENTS AND APPARATUS

1. Sulfuric Acid, 1:1 solution: mix a volume of concentrated sulfuric acid with an equal volume of distilled water.

2. Organic Solvent: Petroleum ether (30-60°C), trichlorotrifluoroethane (47°C) and/or chloroform (61-62°C) may be used (caution petroleum ether is highly flammable).

3. Sodium Sulfate, anhydrous: analytical reagent grade.

4. Separatory Funnel: 1-liter capacity (sufficient capacity for 1-liter of sample plus addition of acid and solvent while still leaving space for proper agitation) with teflon stopcork.

5. Electric Heating Mantle or Steam Bath.

C. STANDARDIZATION -- None

D. PROCEDURE

1. Place a 1000 mℓ sample in a separatory funnel (adequate size) and acidify with 5 mℓ sulfuric acid (1:1) per liter of sample.

2. Rinse the sample bottle with 15 mℓ of petroleum ether and pour the washings into the separatory funnel. Rinse the sample

bottle with an additional 25 mℓ of petroleum ether and pour into the separatory funnel.

3. Shake the separatory funnel vigorously for 2 min. (Caution: vent the pressure after the first few shakes.) Allow the layers to separate (10 min.) and draw off the aqueous layer into the sample container. Place the solvent layer into a 250 mℓ Erlenmeyer flask containing 0.5 - 1.0 g of anhydrous sodium sulfate.

4. Return the sample to the separatory funnel and repeat the rinsing and extraction procedures two additional times, each time add the solvent layer to the 250 mℓ Erlenmeyer flask containing the first petroleum extract.

5. Filter the extract through a plug of grass wool into a tared flask (including a boiling chip) using liberal washings of petroleum ether.

6. Evaporate the solvent nearly to dryness using a steam bath or an electric heating mantle. After cooling, volatilize the last solvent with a gentle stream of air or nitrogen gas.

7. Wipe the sides of the flask and allow to dry in the room for 30 min. Then place in a desiccator for 30 min. to an hour and then determine the weight of the sample.

E. CALCULATIONS

The concentration of material in the sample is calculated as follows:

Extractable organic matter (mg/ℓ)*

$$= \frac{A-B}{C} \times 1000$$

where

A = total weight (receiver + extract), in grams,

B = receiver tare, in grams, and

C = water sample volume, in liters.

*If the organic solvent used for the extraction is not free of residue and not been recently distilled a blank must be run.

REACTIVE (ORTHO) PHOSPHATE

(Ascorbic Acid or Murphy-Riley Technique)

A. GENERAL

1. Reference: Strickland and Parsons, *A Practical Handbook of Seawater Analysis* (1968) pp. 49-52 and *Standard Methods* (1975) pp. 481-483. Revisions of method submitted by Armstrong, U.C. Davis, California.

2. Outline of Method: Reactive phosphorus in the sample is complexed in the presence of molybdic acid, ascorbic acid, and antimony to a blue colored molybdate complex. The intensity of the blue color is measured as absorbance at 885 mμ.

B. SPECIAL REAGENTS

1. Armstrong Reagent: Add 122 mℓ of conc. H_2SO_4 to 800 mℓ of DW. While the solution is still hot, add 10.5 g of Ammonium Molybdate and 0.3 g Antimony potassium tartrate. Heat to dissolve; cool, and dilute to exactly 1 liter with DW, stable indefinitely.

2. Ascorbic Acid Solution: Dissolve 3 g of Ascorbic Acid in 100 mℓ of DW. Note: Stage only one week (store in refrigerator).

C. STANDARDIZATION

1. Conc. Stock Potassium Phosphate: Dry 3-4 g of reagent grade anhydrous K_2HPO_4 at 100 C for 1 hour. Dissolve exactly 2.81 g of the dry salt in DW and dilute to exactly 500 mℓ with DW. Store in plastic bottle (stable for 6 months).

$$1\ m\ell = 1\ mg\ PO_4\text{-}P$$

2. Dilute K_2HPO_4: Dilute 1 mℓ of the conc. solution to 200 mℓ with DW. Make fresh for each standardization procedure.

$$1\ m\ell = 5\ \mu g\ PO_4\text{-}P$$

NOTE: The reagent blank is DW with the reagents added; therefore, the color added by the reagent itself is accounted for in the standard curve.

3. Standard Curve:

0 to 700 µg P/ℓ

4. Procedure for daily standardization

	Concentration
A. Dilute 1 mℓ 1 mg/mℓ → 100 mℓ	1 mg/100 mℓ
	10 mg/ℓ
B. Dilute 1 mℓ 10 µg/mℓ → 100 mℓ	.01 mg/mℓ or 10 µg/mℓ
	10 µg/100 mℓ
	100 µg/ℓ

D. PROCEDURE

1. Measure absorbance of samples without reagents to obtain a turbidity correction factor, if necessary. Always run test on filtered sample only!

2. Measure a 50 mℓ sample into a 150 mℓ beaker.

3. Add 5 mℓ of Armstrong Reagent, and 1 mℓ of Ascorbic Acid; swirl to mix.

4. Allow a 20 min. reaction period (stable up to 2 hrs.), and then read the absorbance against DW at 885 mℓ using a 1 or 5 cm cell.

5. Subtract turbidity correction (if any) from sample absorbance; plug this corrected absorbance into the slope-intercept formula for the standard curve to obtain µg PO_4-P/ℓ.

E. CALCULATION

$$m^{-1} = \frac{\text{concentration}}{\text{absorbance}}$$

d.f. = dilution factor

Sample Concentration µg PO_4-P/ℓ = $(Abs_{sample} - Abs_{blank})(m^{-1})(d.f.)$

If a 10 percent sample is used: d.f. = 10, if a 5 percent sample is used: d.f. = 20.

TOTAL PHOSPHORUS

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 476.
   E.P.A. Methods for Chemical Analysis of Water and Wastes (1971).

2. Outline of Method: The total phosphate content of the sample includes all the orthophosphate, polyphosphates, and most typical organic phosphate compounds that are hydrolyzed to orthophosphate through mild acid hydrolsis. If knowledge about more complete digestion is required, compare method with other digestion techniques (see *Standard Methods*). Polyphosphates do not respond appreciably to the orthophosphate tests alone but are hydrolyzed to orthophosphate by boiling with acid.

   After digestion total phosphate then is measured quantitatively by the method selected for orthophosphate determinations.

B. SPECIAL REAGENTS

1. Strong Acid for Hydrolysis: Slowly add 300 mℓ of conc. H_2SO_4 to approximately 600 mℓ of DW. When cooled, dilute to 1 liter.

2. Phenolphthalein Indicator: Dissolve 2.5 g of phenolphthalein powder into 250 mℓ of ethyl alcohol and dilute to 500 mℓ with DW.

3. Sodium Hydroxide-1N: Dissolve 40 g solid NaOH in DW and dilute to 1 liter with DW.

4. Ammonium Persulfate, $(NH_4)_2S_2O_8$: Small powder scooper (~0.4 g capacity).

5. Armstrong Reagent: See Reactive (Ortho) Phosphate.

6. Ascorbic Acid: See Reactive (Ortho) Phosphate.

7. HCl Solution for Cleaning Glassware: Dilute 10 mℓ conc. to 1 liter with DW.

C. STANDARDIZATION

See Orthophosphate - but subject standard to digestion.

D. PROCEDURE

1. For high precision work it is suggested that glassware in this test be washed without the use of detergent. Also glassware should be segregated and not used interchangeably for high and low phosphorus samples. Wash designated flasks with water only, and then rinse with a dilute solution of HCl. Occasionally, flasks should be allowed to stand 24 hrs. or more with 100 mℓ of the HCl solution in each. Rinse 4-5 times with DW.

2. Add 1 mℓ of the strong-acid solution to a 50 mℓ sample in a 125 mℓ Erlenmeyer flask.

3. Add 0.4 g ammonium persulfate. Cover flask with an inverted beaker.

4. Heat in autoclave for 30 min. at 121°C (15-20 psi).*

5. Add 4-5 drops of phenolphthalein and adjust to pink with 1 N NaOH. Bring back to colorless with one drop of the strong-acid solution. Cool.

6. Dilute to 100 mℓ with DW. This constitutes a 1:2 dilution.

7. Determine orthophosphate using the ascorbic acid technique.

E. CALCULATION

See Orthophosphate.

*NOTE: If suspended material is present in sample at this time, filter sample before neutralization.

TOTAL PHOSPHORUS IN SEDIMENTS

(Persulfate Digestion)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 476.
   Schmalz, K. L. *Phosphorus Districtuion in the Bottom Sediments of Hyrum Reservoir* (1971) pp. 52-54.

2. Outline of Method: Sediment weight (0.15 g) determined from lake samples. This fixed amount of sediment is persulfate digested to measure organic and available inorganic phosphorus.

B. SPECIAL REAGENTS

1. See Total Phosphorus

C. STANDARDIZATION

1. See Total Phosphorus

D. PROCEDURE

1. Weigh out 0.15 g of oven-dry (8-10 hours at 103°C) sediment and place in a 125 mℓ Erlenmeyer flask.

2. Add 50 mℓ distilled water, 1 mℓ strong-acid solution and 0.4 g $(NH_4)_2S_2O_8$. Also run 2 50 mℓ DW blanks.

3. Heat mixture in autoclave for 30 min. at 121°C (15-20 psi).

4. Filter mixture through a GF/C filter; filtrate should be clear.

5. Because of problems with P precipitation upon addition of base, at this point measure out ½ of the filtrate and place in the flask (storing the remaining ½ in the beaker in case you make a mistake) for analysis.

6. Add 4-5 drops of phenolphthalein and adjust filtrate to pink with 1 N NaOH. Bring colorless with one drop of strong-acid solution. Cool and dilute to 100 mℓ. Analyze entire 100 mℓ for PO_4-P; this involves addition of 10 mℓ Armstrong's Reagent and 2 mℓ Ascorbic Acid solution.

7. Take concentration in µg PO_4-P/ℓ directly from standard curve:

$$\mu g\ PO_4\text{-}P/g\ \text{sediment} = (\mu g\ PO_4\text{-}P/\ell)\ 1.33$$

Note: This calculation will only work for above dilution procedure.

E. CALCULATION

0.15 g sediment → 50 mℓ Distilled Water

↓

Autoclave

↓

Filter → Store ½

↓

Neutralize ½ and Dilute to 100 mℓ

↓

Analyze: $Absorbance_{sample}$

$m^{-1} = \frac{\Delta\ \text{concentration}}{\Delta\ \text{absorbance}}$ from ortho-phosphate standard

Concentration µg P/g Sediment

*blank = blank run through procedure

$$= \underbrace{(Abs_{sample} - Abs^{*}_{blank})(m^{-1})}_{\mu g\ P/\ell}\underbrace{(\tfrac{1}{10})}_{\mu g\ P/100\ m\ell\ analyzed}\underbrace{(2)}_{\mu g\ P/sediment\ sample}\underbrace{(\tfrac{1}{0.15})}_{\mu g\ P/g\ sediment}$$

$$\mu g\ P/g\ \text{sediment} = (Abs_{sample} - Abs^{*}_{blank})(m^{-1})(1.333)$$

SOLIDS DETERMINATION

A. Seston; Suspended Solids
B. Rehydrated Volatile Suspended Solids

A. GENERAL

1. Reference: Strickland and Parsons, *A Practical Handbook of Seawater Analysis* (1968) pp. 181-184.
Standard Methods (1975) pp. 94-95

2. Outline of Method: A thoroughly mixed sample is filtered through a tared glass fiber filter; taking the difference between the filter weight and the filter weight plus the suspended material; dividing that difference by the volume of sample used. The result if Suspended Solids in mg/liter.

   Terminology - Seston, method from S&P, filters dried at 75°C.
   - Suspended Solids, method from S-M, filters dried at 103°C.

   The filter is than ashed, rehydrated, dried and weighed, the difference between the last two weighings being Rehydrated Volatile Suspended Solids in mg/ℓ. The filter is rehydrated after ashing to replace water lost from inorganic compounds exposed to 550°C heat.

B. SPECIAL APPARATUS & EQUIPMENT

1. Glass Fiber Filters: Whatman GF/C - 4.25 cm disc size.

2. Semi-Micro Analytical Balance.

3. Drying Oven & Muffle Furnace.

4. Flat-bladed forceps without serrated tips.

5. Filtration Setup: Side arm flask, pump, funnel. A manostat device set to regulate the suction to the filtration unit to prevent the vacuum becoming greater than 300-350 mm Hg.

C. STANDARDIZATION - NONE REQUIRED

D. PROCEDURE - FILTER PREPARATION

1. Wash filters: Place filter on filtration set up and filter

approximately 100 mℓ distilled water. Dry in oven at 103°C, then place in muffle furnace at 550°C for 30 min. Remove, cool to room temperature (in a desiccator) and weigh.

PROCEDURE - SS DETERMINATION (Seston is the same except using a drying temperature of 75°C.)

1. Use smooth forceps at all times to handle filters.

2. Filter as much sample as will easily pass through the filter. Rinse down the funnel with a small amount of DW. Never add so much sample that the filter becomes clogged and some sample has to be discarded from the funnel.

3. Record the Filter Number, Filter Weight, and Sample Volume in liters.

4. Place filters in oven and dry for at least 1 hour at 103°C (usually dried overnight).

5. Weigh the dried filter, which has been cooled to room temperature in a desiccator, and proceed to VSS determination if appropriate.

6. Formula:

$$\text{Suspended Solids} = \frac{\text{Final Weight - Initial Weight}}{\text{Sample Volume in liters}} \text{ in mg/ℓ.}$$

IMPORTANT NOTE: DO NOT EXCEED SUCTION LIMIT OF 15" Hg.

Note: Because filters are hygroscopic and may pick up or lose water from the air, S&P uses a blank value which consists of running 1 or 2 filters along with the samples merely weighing them before the 2 weighing steps obtaining their differences.

$$X = W(2) - W(1)$$

$$SS = \text{Final Wt.} - \text{Initial Wt.} + X/\text{Vol.}$$

PROCEDURE FOR VOLATILE SUSPENDED SOLIDS

1. Place the filter after the second weighing for SS in a crucible and ash for 1 hour at 550°C in the muffle furnace.

2. Allow the crucible to cool to room temperature; rehydrate the filter with a few drops of DW.

3. Place filter (still in crucible) in oven at 103°C for a minimum of 1 hour (usually overnight).

4. Cool to room temperature in a desiccator; weigh the filter.

$$RVSS = \frac{\text{Filter Weight with SS - Rehydrated Ashed Filter Weight}}{\text{Sample Volume Filtered}} \text{ in mg/liter}$$

SOLIDS

A. Total Dissolved
B. Total

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 91-93.
   EPA, *Methods for Chemical Analysis of Water and Wastes* (1976) pp. 266-272.

2. Outline of Method: The filtered or unfiltered sample is evaporated to dryness and the material remaining is total dissolved or total solids, respectively.

B. SPECIAL APPARATUS & EQUIPMENT

1. Glass Fiber Filters: Whatman GF/C - 4.25 cm disc.

2. Drying Oven.

3. Balance.

C. STANDARDIZATION - NONE REQUIRED

D. PROCEDURE

1. Any non-homogeneous materials should be removed from the sample.

2. Choose an aliquot of sample which contains a residue of at least 25 mg.

3. Clean a 250 mℓ beaker (Pyrex) in chromic acid solution, rinse with distilled water, and dry in an oven at 103°C for at least 1 hour.

4. Cool in desiccator and weigh.

5. Measure out aliquot of filtered or unfiltered sample into a beaker; do not handle beaker directly with hands.

6. Place in oven at 98°C and evaporate to dryness.

7. Place in an oven at 103°C for at least 1 hour. { 180°C TDS / 103°C TS

8. Desiccate and weigh.

9. Repeat drying, desiccating, weighing cycle (Step 7-8) until loss of weight is less than 4% of the previous weight, or 0.5 mg, whichever is less.

E. CALCULATION

Total Solids (unfiltered sample)

Total Dissolved Solids (filtered sample)

$$mg/\ell = \frac{\text{Wt. of sample + dish - Wt. of dish}}{\text{Vol. of sample in } \ell}$$

SETTLEABLE MATTER

(By Weight)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 95-96.

2. Outline of Method: This is a measure of the "settling qualities" of suspended solids and measures the portion of material which settles within one hour. It also includes the material which floats to the surface.

B. SPECIAL REAGENTS: None

C. STANDARDIZATION: None

D. PROCEDURE

1. Measure suspended solids (see SS method) in total sample.

2. Place the well mixed sample in a cylinder having $\geq$ 9 cm diameter, $\geq$ 20 cm height, and volume $\geq$ 1300 mℓ.

3. After one hour siphon $\sim$ 250 mℓ of sample from a point halfway between the floatable materials and the settled materials. Do not disturb the surface or bottom material when sampling.

4. Measure suspended solids on this material (the nonsettling matter).

5. mg/ℓ settleable matter = mg/ℓ suspended solids - mg/ℓ nonsettling matter.

E. CALCULATION

mg/ℓ settleable matter = mg/ℓ suspended solids - mg/ℓ nonsettling matter.

SULFATE

(Turbidimetric Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 496-498.

2. Outline of Method: Sulfate ion is precipitated in a hydrochloric acid medium with barium chloride. The absorbance of the resultant barium sulfate suspension is measured spectrophotometrically using a standard curve. Analyzed at room temperature; under strong acid conditions, sulfate is the only ion which will form insoluble compounds in normal waters. The accuracy of the technique decreases for sulfate concentrations above 40 mg/ℓ so dilution with demineralized water may be necessary to obtain the appropriate concentration range.

B. REAGENTS

1. Conditioning Reagent: Mix 50 mℓ glycerol with a solution of 30 mℓ con. HCl, 300 mℓ distilled water, 100 mℓ 95 percent ethyl or isopropyl alcohol, and 75 g sodium chloride.

2. Barium Chloride: Crystals, 20-30 mesh.

C. STANDARDIZATION

1. Stock Sulfate Solution: Dissolve 0.1479 g anhydrous sodium sulfate Na_2SO_4 in distilled water, and dilute to 1 liter.

$$1\ m\ell \equiv 100\ \mu g\ SO_4^{=}$$

Make 100 mℓ standards of 0, 10, 20, 30, and 40 mg/ℓ.

D. PROCEDURE

1. Measure 100 mℓ sample (or an aliquot diluted to 100 mℓ) into a 250 Erlenmeyer flask.

2. Add exactly 5.00 mℓ conditioning reagent.

3. Mix using mag. mix and stirring bar (always set at a constant speed).

4. While solution is stirring, add a spoonful (0.2-0.3 g) $BaCl_2$ crystals and begin timing immediately.

5. Stir exactly 1 min. at the constant speed.

6. At the end of the stirring period, place sample in 5 cm cuvette and measure absorbance at 30 sec. intervals for 4 min. at 420 mμ. Use maximum absorbance obtained within 4 min. period to calculate $SO_4^{=}$.

7. Run a standard sample (20 mg/ℓ) every 3-4 unknowns to insure that conditions are stable. If any turbidity is present, subtract absorbance of untreated sample, then use corrected absorbance to determine $SO_4^{=}$. In other words the untreated sample should be used as the blank.

E. CALCULATION

Calculate concentration of $SO_4^{=}$ in mg/ℓ from the standard curve (over range 0-40 mg/ℓ).

VOLATILE ACIDS (TOTAL ORGANIC ACIDS)

(Column-Partition Chromatographic Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 527-529.

2. Outline of Method: An acidified aqueous sample containing organic acids is passed through a column packed with an inert material, and the organic acids are quantitatively adsorbed onto the material. An organic solvent is then passed through the column and the organic acids are extracted and eluted from the column; they are then measured volumetrically by titration with a standard base.

B. SPECIAL REAGENTS

1. Silicic Acid, 50-200 mesh: Slurry the acid in distilled water and decant and discard the supernatant after 15 minutes of settling. Repeat several times. Dry the acid at 103°C (ABSOLUTELY DRY) and store in desiccator.

2. Chloroform-Butanol Reagent, CB_{25}: Mix 300 mℓ of reagent grade chloroform, 100 mℓ n-butanol, and 80 mℓ of 0.5 N H_2SO_4 in a separatory funnel. Let stand; separate and draw off the lower organic solvent phase and pass through a fluted filter paper into a dry bottle.

3. Thymol Blue Indicator: Dissolve 80 mg thymol blue in 100 mℓ of absolute methanol.

4. Phenolphthalein Indicator: Dissolve 80 mg of phenolphthalein in 100 mℓ of absolute methanol.

5. Sulfuric Acid: Conc. reagent grade.

6. Standard Sodium Hydroxide Titrant, 0.02 N: Dilute 20 mℓ of stock 1.0 N NaOH to 1 liter with absolute methanol.

    A. 1 N NaOH: Dissolve 40 g NaOH in distilled water and dilute to 1 liter.

7. Standard Potassium Biphthalate: Dissolve 4.085 g of anhydrous $KHC_8H_4O_4$ in CO_2 free distilled water and dilute to 1 liter.

C. STANDARDIZATION

1. Dilute 10 mℓ of $KHC_8H_4O_4$ standard to 100 mℓ with CO_2 free water and titrate with 0.02 N NaOH to the phenolphthalein endpoint, 8.3 pH.

$$N_{NaOH} = \frac{0.02\ (10)}{\text{mℓ NaOH titrant used}} = \frac{0.2}{\text{mℓ NaOH}}$$

D. PROCEDURE

1. Centrifuge or vacuum filter the sludge to obtain 10-15 mℓ clear sample.

2. Add 2-3 drops thymol blue indicator and add conc. H_2SO_4, dropwise until definitely red to thymol blue (pH 1.0-1.2).

3. Place 12 g prepared silicic acid into the funnel portion of a side arm filtration flask set up containing a Distilled Water rinsed glass fiber filter. (Place silicic acid on top of the filter and uniformly distribute in narrow, lower section of the funnel.)

4. With a pipet, distribute 5.0 mℓ of acidified sample uniformly over surface of silicic acid "column." Apply suction and draw sample into column. Release suction after last portion of sample has entered the column.

5. Quickly add 65 mℓ CB_{25} reagent and apply suction. Release suction just before the last of the reagent enters the column.

   USE A NEW COLUMN FOR EACH SAMPLE TO BE ANALYZED.

6. Purge the eluted sample with N_2 gas, and continue to do so as the sample is titrated with 0.02 N NaOH to the phenolphthalein endpoint. (Add 2-3 drops of phenolphthalein prior to titration.)

BLANK

Run a blank consisting of 5.0 mℓ acidified (H_2SO_4) distilled water; extract with 65 mℓ CB_{25}, and titrate using the same procedure as was used with the sample.

E. CALCULATION

$$\text{Total Organic Acids (mg/ℓ as acetic acid)} = \frac{(A-B)\ (N)\ (60{,}000)}{\text{mℓ sample}}$$

A = mℓ NaOH titrant used for sample

B = mℓ NaOH titrant used for blank

N = normality of the NaOH titrant

SILICA

(Molybdosilicate Method)

A. GENERAL

1. Reference: *Standard Methods* (1975) pp. 487-490; and EPA, *Methods for Chemical Analysis of Water and Wastes* (1976) pp. 274.

2. Outline of Method: Silica (along with phosphates) will react with ammonium molybdate at approx. pH 1.2 to form heteropoly acids. Oxalic acid is then added to destroy any molybdophosphoric acid present.* (Oxalic acid addition is mandatory.) Run blanks and avoid glass as much as possible. Interferences: Fe, color, turbidity, $S^{=}$ and PO_4.

   *The molybdosilicic acid (yellow color) solution is then measured spectrophotometrically at 410 mμ.

B. SPECIAL REAGENTS

Store reagents in polyethylene.

1. Hydrochloric Acid, 1 + 1: Add equal parts of HCl and distilled water.

2. Ammonium Molybdate Reagent: Dissolve 10 g $(NH_4)_6Mo_7O_{24}\text{-}4H_2O$ in distilled water with stirring and gentle warming and dilute to 100 mℓ. Filter if necessary. Adjust pH to 7-8 with 1 N NaOH to stabilize.

3. Oxalic Acid: Dissolve 10 g $H_2C_2O_4 \cdot 2H_2O$ in distilled water and dilute to 100 mℓ.

C. STANDARDIZATION

1. Prepare 1 mg/mℓ stock using Silica Dilut-it concentrated standard solution

   1 mg = 1 mℓ

2. Dilute 1 → 100

   100 μg ≡ 1 mℓ

D. PROCEDURE

1. To a 50 mℓ sample or an aliquot diluted to 50 mℓ add in rapid succession 1.0 mℓ of 1 + 1 HCl and 2.0 mℓ of the ammonium molybdate solution.

2. Mix by inverting at least six times.

3. Allow solution to stand 5-10 min.

4. Add 1.5 mℓ of the oxalic acid solution and mix thoroughly.

5. Read color after 2 yet before 15 minutes on spectrophotometer at 410 mμ.

E. CALCULATION

1. $m^{-1} = \frac{\Delta \text{ concentration}}{\Delta \text{ absorbance}} = \frac{10 - 0}{Abs_{std} - Abs_{blank}}$

2. $mg/\ell\ SiO_4 = (Abs_{sample} - Abs_{blank})(m^{-1})(d.f)$*

*$d.f$ = 10 if sample volume is 10%
= 20 is sample volume is 5%