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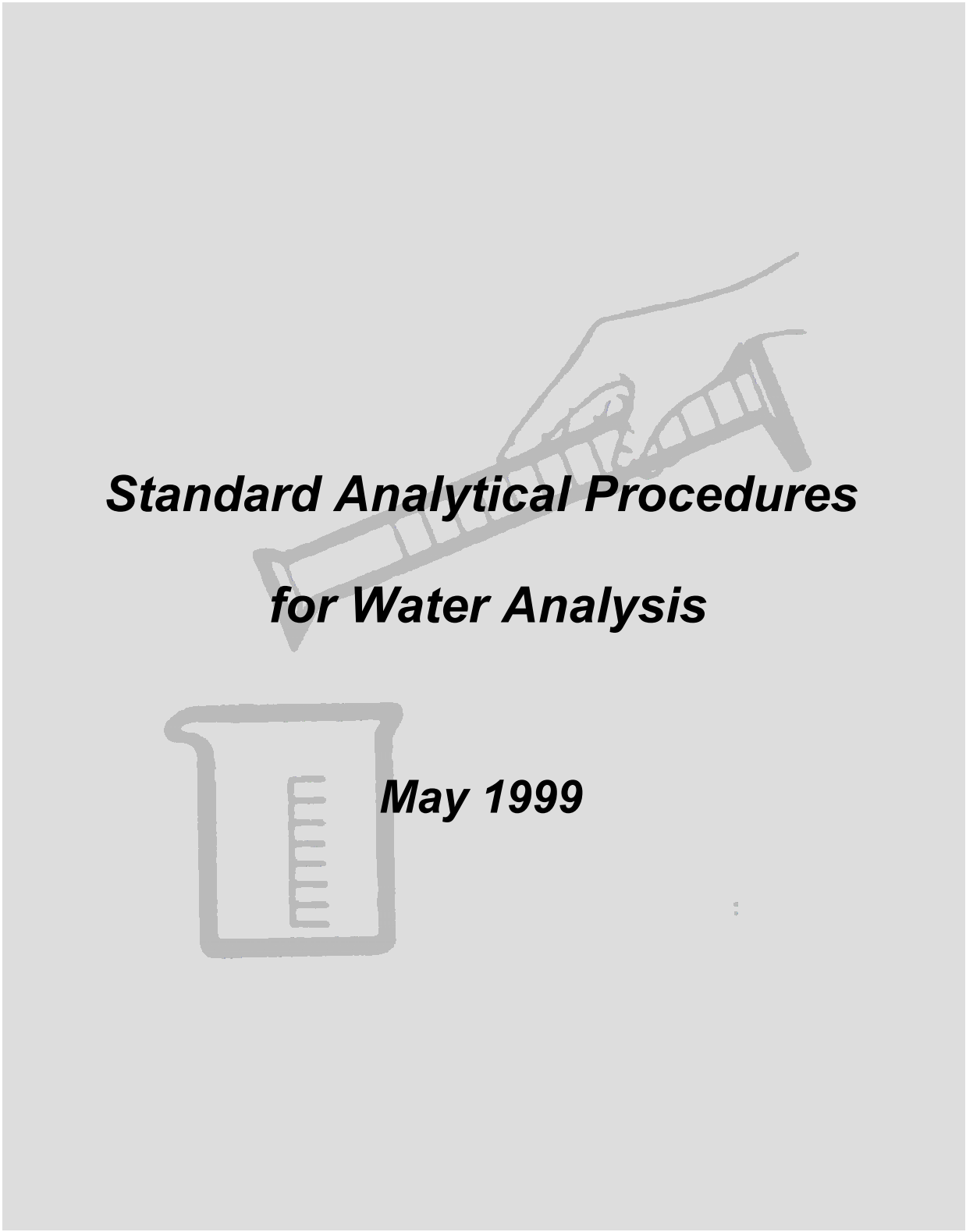

Standard Analytical Procedures for Water Analysis

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01 ANALYTICAL PROCEDURES

Introduction

In the Water Quality Standardisation Workshop, December 9 – 10, 1996, it was recommended that a user friendly Standard Analytical Procedure (SAP) Manual for analysis of water samples should be prepared for use in chemical laboratories under HP. The present SAP manual comprising 38 procedures is based on 'Standard Methods for the Examination of Water and Wastewater' (Standard Methods), 19th edition, APHA, AWWA, WEF, 1995, with one exception as noted below. The reasons for using Standard Methods as a reference are:

- Almost all chemical laboratories under HP are using procedures adopted from older editions of Standard Methods. Only a few are using the 19th edition.

- Standard Methods is also used by other agencies, such as State and Central Pollution Control Board Laboratories, State Public Health Laboratories, Water and Sewerage Boards, etc.

- Methods prescribed by the Bureau of Indian Standards (BIS) are also based on Standard Methods. These were published mostly between 1970 and 1980. The only exception is the procedure recommended by BIS for the determination of biochemical oxygen demand (BOD), which is different from the procedure given in the Standard Methods. In the manual, therefore, the BIS procedure for measurement of BOD (IS 3205 Part 44:1993) is included.

- The Standard Methods is an internationally recognised treatise, which is revised and updated every two to five years.

The draft version (Version 1) of these procedures was sent to the Nodal Officers of various agencies, particpating in HP, for review by their respective laboratory staff and also directly to some laboratories. Comments were received from ten reviewers. All corrections were incorporated in the present version (Version 2). Suggestions were also received for including alternative procedures and expanding the scope of the manual. These could not be included since the purpose of this manual is to give procedures which can easily be followed without increasing its complexity and volume.

The procedures given in the SAP manual can be used for analysis of commonly encountered environmental water samples. The user should refer to the original text for unusual cases, details of interference, precautions, chemistry of reactions and precision and accuracy of the tests.

Alk-P	ALKALINITY, PHENOLPHTHALEIN (PH 8.3)
Method:	**TITRIMETRIC TO PH=8.3 (PHENOLPHTHALEIN)**
ID: ***1.1***	***Version:*** *2*

Apparatus

a. Standard laboratory glassware such as burettes, volumetric flasks, conical flasks and beakers.

Reagents

a. *Standard sodium carbonate, approximately 0.05N.* Dry 3 to 5g sodium carbonate, Na_2CO_3, at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2g to the nearest mg, dissolve in distilled water and make to 1L.

b. *Standard H_2SO_4, approximately 0.1N*. Dilute 2.8 mL conc. sulphuric acid to 1L. Standardise against 40.00 mL 0.05*N* Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 5. Lift out electrodes, rinse into the same beaker and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker and finish titration to pH 4.3. Calculate normality of sulphuric acid:

$$\text{Normality, N} = \frac{\text{A} \times \text{B}}{53.00 \times \text{C}}$$

where:
A = g Na_2CO_3 weighed into the 1L-flask for the Na_2CO_3 standard (see a.)
B = mL Na_2CO_3 solution taken for standardisation titration
C = mL acid used in standardisation titration

c. *Standard sulphuric acid, 0.02N*. Dilute the approximate 0.1*N* solution to 1L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

where:
N = exact normality of the approximate 0.1*N* solution.

d. *Phenolphthalein indicator solution, alcoholic, pH 8.3.* Dissolve 5 g phenolphthalein in 500 mL 95% ethyl alcohol. Add 500 mL distilled water

Procedure

a. Take 25 to 50 mL sample in a conical flask. Add 2 to 3 drops of phenolphthalein indicator. If it turns pink (pH > 8.3), titrate with 0.02 *N* H_2SO_4 to disappearance of the colour. Record mL titrant used.

Calculation

$$\text{Phenolphthalein alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50000}{\text{mL sample}}$$

where:
A = mL titrant used to phenolphthalein end point
N = normality of titrant

Note:

- For turbid/coloured samples, titration can be performed using a pH meter to end point pH value of 8.3.

Alk-T	ALKALINITY, TOTAL (PH 4.5)
Method:	**TITRIMETRIC TO PH=4.5 (METHYL ORANGE)**
ID: ***1.37***	***Version:*** *2*

Apparatus

a. Standard laboratory glassware such as burettes, volumetric flasks and beakers.

Reagents

a. *Standard sodium carbonate, approximately 0.05N.* Dry 3 to 5g sodium carbonate, Na_2CO_3, at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2g to the nearest mg, dissolve in distilled water and make to 1L.

b. *Standard H_2SO_4, approximately 0.1N*. Dilute 2.8 mL conc. sulphuric acid to 1L. Standardise against 40.00 mL 0.05*N* Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 5. Lift out electrodes, rinse into the same beaker and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker and finish titration to pH 4.3. Calculate normality of sulphuric acid:

$$\text{Normality, N} = \frac{A \times B}{53.00 \times C}$$

where:
A = g Na_2CO_3 weighed into the 1L-flask for the Na_2CO_3 standard (see *a*.)
B = mL Na_2CO_3 solution taken for standardisation titration
C = mL acid used in standardisation titration

c. In case potentiometric titration is not possible use bromcresol green indicator to complete the titration.

d. *Standard sulphuric acid, 0.02N*. Dilute the approximate 0.1*N* solution to 1L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

where:
N = exact normality of the approximate 0.1*N* solution.

e. *Bromcresol green indicator, pH 4.5:* Dissolve 100mg bromcresol green sodium salt in 100 mL distilled water

Procedure

a. Add 2 to 3 drops of bromcresol green indicator. Titrate until change in colour (blue to yellow, pH 4.9 to 4.3) is observed. Record total mL titrant used.

Calculation

$$\text{Total alkalinity, mg } CaCO_3/L = \frac{B \times N \times 50000}{\text{mL samle}}$$

where:
B = total mL of titrant used to bromcresol green end point
N = normality of titrant

Note:

For turbid/coloured samples, titration can be performed using a pH meter to end point pH value of 4.5.

Al	ALUMINIUM
Method:	**ERIOCHROME CYANINE R SPECTROPHOTOMETRIC**
ID: ***1.30***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer:* For use at 535ηm with light path of 1 cm or longer.

b. *Glassware:* Treat all glassware with 1 + 1 warm HCl and rinse with aluminium free distilled water.

Reagents

a. *Stock aluminium solution:* Dissolve 8.791 g aluminium potassium sulphate, $AlK(SO_4)_2.12H_2O$, in water and dilute to 1 L.

b. *Standard aluminium solution:* Dilute 10 mL stock aluminium solution to 1000 mL with distilled water; 1.00 mL = 5.00 μg Al. Prepare daily.

c. Sulphuric acid, H_2SO_4, 0.02 *N* and 6 *N*.

d. *Ascorbic acid solution:* Dissolve 0.1 g ascorbic acid in water and make up to 100 mL in a volumetric flask. Prepare fresh daily.

e. *Buffer reagent:* Dissolve 136 g sodium acetate, $NaC_2H_3O_2.3H2O$, in water, add 40 mL 1*N* acetic acid and dilute to 1 L.

f. *Stock dye solution:* Stock solutions can be kept for about one year. Use any of the following products:

- Solochrome cyanine R-200 (Arnold Hoffman & Co., Providence, R.I.) or Eriochrome cyanine (K & K Laboratories, K & K lab. Div., Life Sciences Group, Plainview, N. Y.) Dissolve 100 mg in water and dilute to 100 mL in a volumetric flask. This solution should have a pH of about 2.9.
- Eriochrome cyanine R (Pfaltz & Bauer, Inc., Stamford, Conn.) Dissolve 300 mg dye in about 50 mL water. Adjust pH from about 9 to about 2.9 with 1 + 1 acetic acid (approximately 3 mL will be required). Dilute with water to 100 mL.
- Eriochrome cyanine R (EM Science, Gibbstown, N.J.) Dissolve 150 mg dye in about 50 mL water. Adjust pH from about 9 to about 2.9 with 1 + 1 acetic acid (approximately 2 mL will be required). Dilute with water to 100 mL.

g. *Working dye solution:* Dilute 10.0 mL of stock dye solution to 100 mL in a volumetric flask with water. Stable for at least 6 months.

h. *Bromcresol green indicator, pH 4.5, solution:* Dissolve 100 mg bromcresol green, sodium salt, in 100 mL distilled water.

i. *EDTA* (sodium salt of ethylenediamine-tetraacetic acid dihydrate), 0.01 M: Dissolve 3.7 g in water and dilute to 1 L.

j. *Sodium hydroxide,* NaOH, 1*N* & 0.1*N*.

Procedure

a. Prepare standards between 0 to 7 μg by taking 0,1.0, 3.0, 5.0 and 7.0 mL standard aluminium solution in 50 mL volumetric flasks and adding water to a total volume of 25 mL

b. Add 1 mL 0.02 *N* H_2SO_4 to each standard and mix. Add 1mL ascorbic acid solution and mix. Add 10 mL buffer solution and mix. With a volumetric pipette add 5.00 mL working dye reagent and mix. Immediately make up to 50 mL with distilled water, mix and let stand for 5 to 10 minutes.

c. Read absorbance at 535 nm within 15 min of addition of dye, adjusting instrument to zero absorbance with the standard containing no aluminium. Plot calibration curve between absorbance and aluminium concentration.

d. Place 25 mL sample, or an aliquot diluted to 25 mL, in a flask, add a few drops of bromcresol green and titrate with 0.02 N H_2SO_4 to yellowish end point. Record reading and discard sample.

e. To two similar samples add the same amount of acid used in the titration and add 1 mL in excess.

f. To one sample add 1 mL EDTA to complex any aluminium present. This will serve as blank. To both samples add 1 mL ascorbic acid, 10 mL buffer reagent, 5 mL working dye reagent , make up to 50 mL and read absorbance as in *c* above.

Calculation

Read aluminium concentration in the sample against its absorbance value from the calibration curve.

Note

An underestimation of the aluminium concentration between 10 and 50% occurs when this method is applied on samples that contain fluoride in the range of 0.4 and 1.5 mg/L.

HCO3	BICARBONATE
Method:	**CALCULATION FROM pH AND ALKALINITY**
ID: ***1.32***	***Version:*** *2*

Standard Analytical Procedure

cedure

Obtain measured values of pH (method 1.21), phenolphthalein alkalinity (method 1.1) and total alkalinity (method 1.37) and calculate.

culation

In case total dissolved solids < 500 mg/L

$$HCO_3^- \text{ as mg } CaCO_3/L = \frac{T\text{-}5.0 \times 10^{(pH-10)}}{1 + 0.94 \times 10^{(pH-10)}}$$

where:

T = total alkalinity as mg $CaCO_3$/L

The above calculations are based on ionisation constants of carbonic acid at 25 ^oC assuming the activity coefficient as 1 and therefore can be used where total dissolved solids are less than 500 mg/L.

In case total dissolved solids > 500 mg/L

Calculate bicarbonate from phenolphthalein alkalinity, P and total alkalinity, T (both mg $CaCO_3$/L) as follows:

Alkalinity result	Bicarbonate, mg $CaCO_3$/L
P=0	T
P<½T	T-2P
P=½T	0
P>½T	0
P=T	0

BOD3-27	BIOCHEMICAL OXYGEN DEMAND (3 DAYS, 27°C)
Method:	**BOTTLE INCUBATION FOR 3-DAYS AT 27°C**
ID: ***1.2***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. BOD bottles, 300 mL, narrow mouth, flared lip, with tapered and pointed ground glass stoppers.

b. Air incubator or water bath, thermostatically controlled at 27 ± 1°C. Light entry must be prevented in order to avoid photosynthetic oxygen production

c. Accessories: plastic tube, screw-pin and a 5-10 L water container.

Reagents

a. *Phosphate buffer solution*. Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl in 1L distilled water.

b. *Magnesium sulphate solution.* Dissolve 22.5 g $MgSO_4.7H_2O$ in 1L distilled water.

c. *Calcium chloride solution.* Dissolve 27.5 g $CaCl_2$ in 1L distilled water.

d. *Ferric chloride solution.* Dissolve 0.25 g $FeCl_3.6H_2O$ in 1L distilled water.

e. *Acid and alkali solution.* 1*N* NaOH and 1*N* H_2SO_4. Use for neutralising samples.

f. *Glucose-glutamic acid solution (prepare fresh).* Dissolve 150 mg dry reagent grade glucose and 150 mg dry reagent grade glutamic acid in 1L distilled water

g. *Sample dilution water*. Add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions per litre distilled water.

Procedure

a. Prepare required amount of dilution water at the rate of 1000 to 1200 mL per sample per dilution. Bring the diluted water temperature to 27°C. Saturate with air by shaking in a partially filled bottle, by bubbling with organic free filtered air or by storing in cotton-plugged bottles for a day.

b. Some samples do not contain sufficient microbial population (for example, some industrial wastes, high temperature wastes, or wastes with extreme pH values). For such wastes, the dilution water is seeded using effluent from a biological treatment system processing the waste. Where this is not available, use supernatant from domestic wastewater after settling for at least 1 h but not more than 36 h. Seed from a surface water body receiving the waste may also be suitable. Add enough seed volume such that the DO uptake of the seeded dilution water is between 0.6 and 1.0 mg/L. For domestic wastewater seed, usually 4 to 6 mL seed / L of dilution water is required. Surface water samples usually do not require seeding.

c. Dilution of sample. Dilutions must result in a sample with a residual DO (after 3 days of incubation) of at least 1 mg/L and a DO uptake of at least 2 mgl/L. Make several dilutions using the Table and experience with the particular sample source. Polluted surface waters may have 5 to 25 mg/L BOD

Using percent mixture		***By direct pipetting into 300mL bottles***	
Range of BOD	% mixture	Range of BOD	mL Sample
1,000 - 3,500	0.2	1,200 - 4,200	0.5
400 - 1,400	0.5	600 - 2,100	1.0
200 - 700	1.0	300 - 1,050	2.0
100 - 350	2.0	120 - 420	5.0
40 - 140	5.0	60 - 210	10.0
20 - 70	10.0	30 - 105	20.0
10 - 35	20.0	12 - 42	50.0
4 - 14	50.0	6 - 21	100.0
0 - 7	100.0	0 - 7	300.0

Table Dilutions for varying BOD values

For preparing dilution in graduated cylinders, siphon dilution water, seeded if necessary, into a 1 to 2 L capacity cylinder. Siphoning should always be done slowly without bubbling, use a screw-pin on the tube to regulate the flow. Keep the tip of the tube just below the water surface as it rises. Fill cylinder half full, add desired quantity of sample and dilute to appropriate level, mix with plunger type mixing rod. Siphon mixed diluted sample in three BOD bottles, stopper without entraining any air. Determine initial DO (method 1.9) on one bottle and incubate the other two at 27°C. Determine final DO (method 1.9) in duplicate after 3days.
For direct pipetting, siphon the desired sample volume to individual bottles and fill with enough dilution water. Complete the test as in the earlier case.

d. Dilution water blank. Find the DO consumption of unseeded dilution water by determining initial and final DO as in c above. It should not be more than 0.2 mg/L

e. Seed control. Determine the DO uptake by seeding material according to the procedure in *c* above.

Calculation

a. When dilution water is not seeded:

$$BOD_{3,27}, mg.l^{-1} = \frac{D_0 - D_T}{P}$$

b. When dilution water is seeded:

$$BOD_{3,27}, mg.l^{-1} = \frac{(D_0 - D_T) - f \times (B_0 - B_T)}{P}$$

where:
D_0 = DO of diluted sample initially, mg/L
D_T = DO of diluted sample after 3 day incubation at 27°C, mg/L
P = decimal volumetric fraction of sample used

B_0 = DO of seed control initially, mg/L
B_T = DO of seed control after incubation, mg/L
f = ratio of %seed in diluted sample to %seed in seed control

Notes:

- Report average results of duplicates if both dilutions are correct.
- Formula does not correct for BOD of dilution water which is only valid for dilution water meeting the criteria. BOD of dilution water should not be more than 0.2 mg/L, preferably lower than 0.1 mg/L.
- The standard glucose-glutamic acid should have BOD of198 ± 37 mg/L (BIS3025 (part 44): 1993). Check procedure otherwise.
- Report BOD values lower than 0.5mg/L or 2 times the measured BOD of the dilution water (whichever is lower) as lower than detection limit.

B	BORON
Method:	**CURCUMIN SPECTROPHOTOMETRIC**
ID: ***1.3***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. Spectrophotometer, or photometer with a green filter, for use at 540nm.

b. High-silica glass or porcelain evaporating dishes, 100 - 150 mL

c. Water-bath, set at 55^0C

d. Glass-stoppered volumetric flasks, 25 - 50 mL capacity.

e. Ion- exchange column, 1.3cm diameter, 50 cm length.

f. Containers, boron free or polyethylene.

Reagents

a. Stock boron solution: Dissolve 571.6mg anhydrous boric acid, in distilled water and dilute to 1L, 1 mL = 100μg B.

b. Standard Boron solution: Dilute 10 mL stock boron solution to 1L with distilled water; 1 mL = 1μg B.

c. Curcumin reagent: Dissolve 40mg finely ground curcumin and 5g oxalic acid in 80 mL 95% ethyl alcohol, add 4.2 mL conc. HCl, make to 100 mL with ethyl alcohol - store in refrigerator (stable for several days).

d. Ethyl alcohol, 95%.

e. Strongly acidic cation exchange resin.

f. Hydrochloric acid, HCl, 1 + 5

Procedure

a. Preparation of calibration curve: Take 0, 0.25, 0.50, 0.75 and1 mL boron standard solution into same size of evaporating dishes, make volume to 1 mL with distilled water, add 4 mL curcumin reagent to each, mix. Heat the dishes on water bath at 55 ± 2°C for 80 min, cool, add 10 mL 95% ethyl alcohol and mix the red coloured product with a polythene rod.

b. Use 95% ethyl alcohol to transfer the dish contents to 25 mL volumetric flasks, make up to the mark with 95% alcohol and mix.

c. Sample treatment: For water expected to have 0.1 - 1mg B/L, use 1 mL sample. For higher concentrations take appropriate sample to make dilutions to 1 mL with distilled water. Run the sample with the standard and blank.

d. Removal of hardness and cation interference for samples containing more than 100mg /L hardness as $CaCO_3$. Use a column with strongly acidic cation-exchange resin, backwash with distilled water, pass 50 mL 1 + 5 HCl at a rate of 0.2 mL acid/mL resin in

column/min. Wash column free of acid with distilled water. Add 25 mL sample to resin column, adjust flow to 2 drops/s and collect in 50 mL, volumetric flask and wash column with distilled water to make up the volume. Alternatively, filter the final solution in step ‘b’ above if any turbidity appears due to hardness of the sample.

e. Make photometric measurements at 540nm.

Calculation

Plot calibration curve giving absorbance versus □g B. Read weight of Boron in □g in the sample from the curve. Calculate mg B/L by dividing the weight by the volume of the sample in mL.

Ca	CALCIUM
Method:	**EDTA TITRIMETRIC**
ID: ***1.29***	***Version:*** *2*

ngents

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

b. *Murexide (ammonium purpurate) indicator*: Mix 200 mg dye with100 g solid NaCl. Grind to 40 to 50 mesh size.

c. *Standard EDTA titrant, 0.01M*: Weigh 3.723 g di-sodium salt of EDTA, EDTA dihydrate, dissolve in distilled water and dilute to 1000 mL. Store in polyethylene bottle, 1 mL = 400.8 μg Ca. Standardise EDTA against standard calcium solution periodically following the method described below.

d. *Standard calcium solution:* Weigh 1.000 g anhydrous $CaCO_3$ in 500 mL flask (primary standard). Add 1 + 1 HCl in small amounts through a small funnel till all $CaCO_3$ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool and add a few drops of methyl red indicator and adjust to intermediate orange colour by adding 3*N* NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 400.8 μg Ca.

cedure

a. Take 50 mL sample or an aliquot diluted to 50 mL such that the calcium content is not more than 10 mg. Samples which contain alkalinity greater than 300 mg/L should be neutralised with acid, boiled for 1 min and cooled before titration.

b. Add 2 mL NaOH solution or a volume sufficient to produce a pH of 12 to 13. Start titration immediately after addition of the alkali. Add 0.1 to 0.2 g indicator mixture. Titrate with EDTA solution, with continuous mixing, till the colour changes from pink to purple. Check end point by adding 1 to 2 drops excess titrant to make certain that no further colour change occurs.

Calculation

$$\text{mg Ca/L} = \frac{A \times B \times 400.8}{\text{mL sample}}$$

where:

A = mL titrant for sample

B = $\frac{\text{mL of standard calcium solution taken for titration}}{\text{mL EDTA titrant}}$

CO3	CARBONATE
Method:	**CALCULATION FROM pH AND ALKALINITY**
ID: ***1.31***	***Version:*** *2*

cedure

a. Obtain measured values of pH (method 1.21), phenolphthalein alkalinity (method 1.1) and total alkalinity (method 1.37) and calculate.

culation

In case total dissolved solids < 500 mg/L:

$$HCO_3^- \text{ as mg } CaCO_3^- = \frac{T - 5.0 \times 10^{(pH-10)}}{1 + 0.94 \times 10^{(pH-10)}}$$

$$CO_3^{2-} \text{ as mg } CaCO_3/L = 0.94 \times HCO_3^- \times 10^{(pH-10)}$$

where:
T = total alkalinity (Alk-T) as mg$CaCO_3$/L
HCO_3^- = bicarbonate

The above calculations are based ionisation constants of carbonic acid at 25 ^oC assuming the activity coefficient as 1 and therefore can be used where total dissolved solids are less than 500 mg/L.

In case total dissolved solids > 500 mg/L

Calculate carbonate from phenolphtalein alkalinity, P and total alkalinity, T (both mg $CaCO_3$/L) as follows:

Alkalinity result	Carbonate, mg $CaCO_3$/L
P=0	0
P<½T	2P
P=½T	2P
P>½T	2(T-P)
P=T	0

COD	CHEMICAL OXYGEN DEMAND
Method:	**OPEN REFLUX**
ID: ***1.4***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. *Reflux flasks*, consisting of 250 mL flask with flat bottom and with 24/29 ground glass neck

b. *Condensers*, 24/29 and 30 cm jacket Leibig or equivalent with 24/29 ground glass joint, or air cooled condensers, 60 cm long, 18 mm diameter, 24/29 ground glass joint.

c. *Hot plate* or gas burner having sufficient heating surface.

Reagent

a. *Standard potassium dichromate solution, 0.0417M (0.25N)*: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103°C for 2 hours, in distilled water and dilute to 1L.

b. *Sulphuric acid reagent*: Add 5.5g Ag_2SO_4 technical or reagent grade, per kg of conc. H_2SO_4, keep for a day or two to dissolve.

c. *Ferroin indicator solution*: Dissolve 1.485g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100 mL. Commercial preparation may also be available.

d. *Standard ferrous ammonium sulphate (FAS), titrant, 0.25M*: Dissolve 98g Fe $(NH_4)_2(SO_4)_2.6H_2O$ in distilled water, add 20 mL conc. H_2SO_4, cool and dilute to 1L, standardise daily as follows.

e. Standardisation: Dilute 10 mL standard $K_2Cr_2O_7$ to about 100 mL, add 30 mL conc H_2SO_4, cool. Add 2 drops of ferroin indicator and titrate with FAS.

f. $$\text{Molarity FAS} = \frac{\text{Volume of } 0.0417\text{M } K_2Cr_2O_7, \quad \text{mL}}{\text{Volume of FAS used}, \quad \text{mL}} \times 0.25$$

g. *Mercuric Sulphate, $HgSO_4$*, powder

h. *Potassium hydrogen phthalate (KHP) standard*: Lightly crush and dry potassium hydrogen phthalate ($HOOCC_6H_4COOK$), at 120°C, cool in desiccator, weigh 425 mg in distilled water and dilute to 1L. This solution has a theoretical COD of 500 μgO_2/mL, stable for 3 months in refrigerator.

Procedure

a. Add 50 mL of sample or an aliquot diluted to 50 mL with distilled water in a 500 mL refluxing flask. Add 1g $HgSO_4$, few glass beads, and 5 mL sulphuric acid reagent, mix, cool. Add 25 mL of 0.0417M $K_2Cr_2O_7$ solution, mix. Connect the flask to the condenser

and turn on cooling water, add additional 70 mL of sulphuric acid reagent through open end of condenser, with swirling and mixing.

b. Reflux for 2 hours; cool, wash down condenser with distilled water to double the volume of contents, cool.

c. Add 2 drops of Ferroin indicator, titrate with FAS the remaining potassium dichromate, until a colour change from bluish green to reddish brown. Also reflux and titrate a distilled water blank with reagents.

d. Use standard 0.00417M $K_2Cr_2O_7$, and 0.025M FAS, when analysing very low COD samples.

e. Evaluate the technique and reagents by conducting the test on potassium hydrogen phthalate solution.

f. Do not add grease at the Leibig jacket to prevent jamming, use water instead.

Calculation

$$\text{COD, mgO}_2\text{/l} = \frac{(\text{A} - \text{B}) \times \text{M} \times 8000}{\text{ml sample}}$$

where:

A = FAS used for blank, mL
B = FAS used for sample, mL
M = Molarity of FAS

Note

- Theoretically the method is suitable for analysing samples containing 1000 mg/L COD without dilution
- In order to economise on quantities of chemicals used in the test, use smaller sample volumes and proportionally reduce quantities of chemicals as given in the following table.

Sample size	Standard potassium dichromate	H_2SO_4 with Ag_2SO_4	$HgSO_4$	Ferrous ammonium sulphate	Final volume before titration
mL	mL	mL	g	mole/L	mL
10.0	5.0	15	0.2	0.05	70
20.0	10.0	30	0.4	0.10	140
30.0	15.0	45	0.6	0.15	210
40.0	20.0	60	0.8	0.20	280
50.0	25.0	75	1.0	0.25	350

Cl	CHLORIDE
Method:	**ARGENTOMETRIC TITRATION**
ID: ***1.33***	***Version:*** *1*

Standard Analytical Procedure

Reagents

a. *Potassium chromate indicator solution:* Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

b. *Standard silver nitrate titrant, 0.0141M (0.0141N):* Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL; 1 mL = 500 μg Cl^-. Store in brown bottle.

Standardise against 10 mL standard NaCl diluted to 100 mL, following the procedure described for the samples.

$$N = 0.0141 \times \frac{mL\ \ standard\ \ NaCl}{V - B}$$

where:

N = normality of $AgNO_3$
V = mL $AgNO_3$ titrant
B = mL titration for blank

c. *Standard sodium chloride, 0.0141M (0.0141N)*: Dissolve 824.0 mg NaCl (dried at 140 °C) in distilled water and dilute to 1000 mL; 1mL = 500 μg Cl^-

d. *Aluminium hydroxide suspension:* Dissolve 125 g aluminium potassium sulphate or aluminium ammonium sulphate, $AlK(SO_4)_2.12H_2O$ or $AlNH_4(SO_4)_2.12H_2O$, in 1L distilled water. Warm to 60°C and add 55mL concentrated ammonium hydroxide slowly with stirring. Let stand for about 1h, transfer to a large bottle. Wash precipitate two times or till free of chloride, by successive addition of distilled water, settling and decanting.

cedure

a. Use a 100 mL sample or a suitable portion diluted to 100 mL. If the sample is coloured or turbid, add 3 mL $Al(OH)_3$ suspension, mix, let settle and filter.

b. Add 1 mL K_2CrO_4 indicator solution, titrate with $AgNO_3$ titrant to a pinkish yellow end point.

c. Repeat the titration with distilled water blank. A blank of 0.2 to 0.3 mL is usual.

culation

$$mg\ \ Cl^-/L = \frac{(A - B) \times N \times 35\ \ 450}{mL\ \ sample}$$

where:

A = mL titration for sample
B = mL titration for blank
N = normality of $AgNO_3$

Chlf-a	CHLOROPHYL-A
Method:	**ACETONE EXTRACTION SPECTROPHOTOMETRIC**
ID: ***1.5***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Spectrophotometer

b. Cuvettes, with 1, 4, and 10cm path lengths

c. Tissue grinder

d. Clinical centrifuge

e. Centrifuge tubes, 15 mL graduated, screw cap

f. Filtration equipment; glass fibre or membrane filters (GFF), 0.45 μm porosity, 47-mm diameter; vacuum pump; solvent resistant disposable filter assembly, 1.0 μm pore size, 10 mL solvent resistant syringe.

Reagents

a. *Saturated magnesium carbonate solution*, add 1g finely powdered $MgCO_3$ in 100 mL distilled water.

b. *Aqueous acetone solution*: Mix 90 parts acetone with 10 parts saturated magnesium carbonate solution.

c. *Hydrochloric acid, HCl, 0.1N*

Procedure

a. Chlorophyll extraction procedure: Concentrate the sample immediately after collection by centrifuging or filtering through GFF. If extraction is delayed, protect from light; keep at 4°C. Samples with pH>7.0 can be kept in opaque plastic bottles, stored frozen for 3 weeks; acidic samples must be processed immediately to prevent degradation of chlorophyll.

b. Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, macerate at 500 rpm for 1 min. Use TFE/glass grinder for GFF.

c. Transfer sample to screw-cap centrifuge tube, rinse grinder with a few mL of 90% aqueous acetone, add the rinse to extracted slurry, avoid excessive dilution, make to 10 mL with 90% aqueous acetone, keep for 2h at 4°C in the dark. GFF, 25 and 47 mm introduce error; 0.3 and 1%, if a 10 mL extraction used.

d. Centrifuge in closed tubes for 20 min at 500g, decant into clean, calibrated, 15 mL screw cap centrifuge tube and note the volume.

e. Transfer 3 mL clarified extract to a 1cm cuvette and read optical density (OD) at 750 and 664nm. Acidify extract in the cuvette with 0.1 mL 0.1*N* HCl, gently agitate the acidified extract and read OD at 750 and 665nm, 90s after acidification.

f. The OD 664 before acidification should be between 0.1 and 1.0. For very dilute extracts use cuvettes having a longer path length. If a larger cell is used, add a proportionately larger volume of acid. Correct OD obtained with larger cuvettes to 1cm before making calculations.

Calculation

- Subtract the 750nm OD values from the readings before acidification (OD 664nm), and after acidification (OD 665nm).
- Using he corrected values calculate chlorophyll a.

$$\text{Chlorophyl}-\text{a},\quad \text{mg/m}^3 = \frac{26.7 \times (664_b - 665_a) \times V_1}{V_2 \times L^{/}}$$

where:
V_1 = Volume of extract, L
V_2 = Volume of sample, m^3
$L^{/}$ = light path or width of cuvette, cm
664_b = corrected optical density of 90% acetone extract before acidification.
665_a = corrected optical density of 90% acetone extract after acidification.

The value 26.7 is the absorbance correction and equals A x K

where:
A = absorbance coefficient for chlorophyll a, at 664nm = 11.0
K = ratio expressing correction for acidification, 2.43

Apparatus

a. As needed for total coliform test

b. Water bath to maintain 44.5 ± 0.2°C

Reagents

a. EC medium

Tryptose or trypticase	20.0 g
Lactose	5.0 g
Bile salts mixture or bile salts No. 3	1.5 g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0 g
Potassium dihydrogen phosphate, KH_2PO_4	1.5 g
Sodium chloride, NaCl	5.0 g
Distilled water	1L

b. Add ingredients to water, mix thoroughly and heat to dissolve. pH should be 6.9 ± 0.2 after sterilisation. Before sterilisation dispense sufficient medium (approximately 10 mL) in fermentation tubes, in which inverted vials are placed (to cover the vial at least two-thirds after sterilisation, it may be completely submerged also). Close tubes with caps and sterilise.

Procedure

a. Carry out presumptive phase as described in the Total Coliform procedure.
b. Subject all positive presumptive tubes to the faecal Coliform test. Gently shake the positive tubes to re-suspend growth and with a sterile loop transfer a loopful to a fermentation tube containing EC medium.
c. Incubate the inoculated tubes at 44.5 ± 0.2°C for 24 ± 2 hours.
d. Gas production with growth within 24 ± 2 hours constitutes a positive reaction.

Calculation

On the basis of the number of positive EC medium fermentation tubes read the bacterial density as in the test for total Coliforms.

TCOL-MPN	COLIFORMS, TOTAL
Method:	**STANDARD MULTIPLE TUBE FERMENTATION**
ID: ***1.7***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Autoclave, for operation at 121°C.

b. Steriliser oven, to maintain 160 -170°C

c. Incubator, to maintain 35 ± 0.5°C

d. Glassware: fermentation tubes 30 - 40 mL capacity with aluminium caps, vials 0.25 - 0.5 mL capacity, pipettes 10 and 1 mL with 0.1 mL graduations.

e. Inoculating wire loop: 22 - 24 gauge nickel alloy wire loop 3 - 3.5 mm diameter for flame sterilisation.

Reagents and Culture medium:

a. *Dilution water*: Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 mL distilled water and adjust to pH 7.2 ± 0.5 with 1*N* sodium hydroxide and dilute to 1L. Distribute at the rate of 9 mL/tube. Close tubes with caps and sterilise.

b. *Lauryl tryptose broth*:

Tryptose	20.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate, K_2HPO_4	2.75 g
Potassium dihydrogen phosphate, KH_2PO_4	2.75
Sodium chloride, NaCl	5.0 g
Sodium lauryl sulphate	0.1 g
Distilled water	1L

Add ingredients to water, mix thoroughly and heat to dissolve. pH should be 6.8 $\pm$ 0.2 after sterilisation. Before sterilisation, dispense sufficient medium (approximately 10 mL) in fermentation tubes, in which inverted vials are placed (to cover the vial at least two-thirds after sterilisation, it may be completely submerged also). Close tubes with caps and sterilise.

c. *Brilliant green lactose bile broth*:

Peptone	10.0 g
Lactose	10.0 g
Oxgall	20.0 g
Brilliant green	0.0133 g
Distilled water	1 L

Prepare, dispense and sterilise as in *b* above.

Note:

- Pre-formed dry powder medium available commercially for both b and c may be used.
- Double the strength of medium if 10 mL inoculum is used.

Procedure

a. Sterilisation: Sterilise culture broths and dilution water, in autoclave at 121^{o}C for 15 min. and pipettes in metal containers in steriliser oven at 170^{o}C for 2h.

b. Presumptive phase:

•Aseptically transfer appropriate sample volumes of the sample to lauryl tryptose fermentation tubes. Sample volume and number of tubes to be inoculated will depend on the quality of water samples. Use the following table as a guide:

Source	Inoculum
Drinking Water	10 mL aliquots inoculated in 10 tubes
Non-potable water, rivers, open wells and tanks	10, 1 and 0.1 mL aliquots inoculated in 5 tubes each
Non-potable and polluted waters	1, 0.1 and 0.01 mL aliquots inoculated in 5 tubes each. For grossly polluted waters it may be necessary to inoculate even smaller volumes.

•For transferring volumes less than 0.1 mL, prepare serial dilutions. To prepare serial dilutions add one mL sample to a dilution tube containing 9 mL dilution water. Mix and transfer one mL from the first dilution to second dilution tube to given second dilution and so on. Thus one mL each of the first dilution, second dilution etc., will represent 0.1 mL, 0.01 mL etc., of the original sample, respectively. Note that the volumes of inocula in a series always decrease by a factor of 10.

•Incubate the inoculated tubes at 35 ± 0.5^{0}C. After 24 ± 2h swirl each tube and examine for gas production. If no gas is evident, re-incubate and re-examine at the end of total 48 ± 3h. Record presence or absence of growth and gas. Presence of both gas and growth constitutes a positive presumptive test.

c. Confirmed phase:

- Subject all positive presumptive tubes to the confirmed phase. Gently shake the positive tubes to re-suspend growth and with a sterile loop, transfer one loopful to a fermentation tube containing brilliant green lactose bile broth.
- Incubate the inoculated tubes at 35 $\pm$ 0.5^{o}C. Formation of gas within 48 $\pm$ 3h constitutes a positive confirmed phase.

Calculation

1. *When ten tubes of 10mL each are incubated read from Table 1*

   On the basis of the number of positive tubes in the confirmed phase read the bacterial density for samples for which 10 tubes of 10 mL aliquots were inoculated from Table 1

2. *When three test dilutions are incubated read from Table 2*

   On the basis of the number of positive tubes in the confirmed phase read the bacterial density for samples for which 10, 1.0 and 0.1 mL aliquots were inoculated each in 5

tubes from Table 2. For smaller inocula also use Table 2 but multiply the values by an appropriate factor, e.g. 10 for 1, 0.1, 0.01 mL inocula series and 100 for 0.1, 0.01, 0.001 mL inocula series.

3. *For the combination of positive tubes not appearing in Table 2 use the following formula*:

$$\text{MPN/100 mL} = \frac{\text{no. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

4. *When more than three test dilutions are incubated, the following rules are used in determining MPN value (see Table 3 for a worked out example):*

   4.1. Choose the highest dilution that gives positive results in all five portions tested and the two next higher dilutions.

   4.2. Where positive results occur in dilutions higher than the three chosen according to the above rule (4.1), they are incorporated in the results of the highest chosen dilution up to a total of five.

   4.3. If only one dilution gives a positive result, two dilutions immediately lower and higher giving zero positives should be chosen so as to keep the positive result in the middle of the series.

Note:

- Most likely positive combinations are given in Table 2. If unlikely combinations occur with a frequency greater than 1% it is an indication that the technique is faulty.

No of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index / 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	< 1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	> 23.0	13.5	Infinite

Table 1 MPN Index and 95% Confidence Limits for Various Combinations of Positive and Negative Results when Ten 10 mL Portions are used

Combination of Positives	MPN Index / 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0-0-0	< 2	-	-
0-0-1	2	1.0	10
0-1-0	2	1.0	10
0-2-0	4	1.0	13
1-0-0	2	1.0	11
1-0-1	4	1.0	15
1-1-0	4	1.0	15
1-1-1	6	2.0	18
1-2-0	6	2.0	18
2-0-0	4	1.0	17
2-0-1	7	2.0	20
2-1-0	7	2.0	21
2-1-1	9	3.0	24
2-2-0	9	3.0	25
2-3-0	12	5.0	29
3-0-0	8	3.0	24
3-0-1	11	4.0	29
3-1-0	11	4.0	29
3-1-1	14	6.0	35
3-2-0	14	6.0	35
3-2-1	17	7.0	40
4-0-0	13	5.0	38
4-0-1	17	7.0	45
4-1-0	17	7.0	46
4-1-1	21	9.0	55
4-1-2	26	12	63
4-2-0	22	9.0	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9.0	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360
5-3-3	170	80	410
5-4-0	130	50	390
5-4-1	170	70	480
5-4-2	220	100	580
5-4-3	280	120	690
5-4-4	350	160	820
5-5-0	240	100	940
5-5-1	300	100	1300
5-5-2	500	200	2000
5-5-3	900	300	2900
5-5-4	1600	600	5300
5-5-5	≥ 1600	-	-

Table 2. MPN Index and 95% Confidence Limits for Various Combinations of Positive Results with Five Tubes per Dilution (10 mL, 1.0 mL, 0.1 mL)

Ex.No	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL	0.0001 mL	MPN index /100mL	MPN /100mL
1.	5/5	3/5	1/5	-	-	-	110	110
2.	4/5	3/5	5/5	-	-	-		46
3.	5/5	5/5	5/5	3/5	2/5	1/5	170	17000
4.	5/5	5/5	5/5	5/5	3/5	2/5	140	140000
5.	5/5	5/5	0/5	3/5	2/5	1/5		471
6.	5/5	5/5	5/5	4/5	3/5	1/5	350	35000
7.	-	5/5	5/5	2/5	0/5	-	50	5000
8.	-	5/5	4/5	2/5	0/5	-	220	220
9.	0/5	1/5	0/5	0/5			2	2
10.	-	5/5	3/5	1/5	1/5	-	140	140

Table 3 Examples for reading positive tubes: regular reading of Table I (no 1), using the formula (no 2 and 5) and dealing with more than three test dilutions (no 3-10).

COL	COLOUR
Method:	**VISUAL COMPARISON**
ID: ***1.8***	***Version:*** *1*

Apparatus

a. *Nessler tubes*: Matched, 50 mL, tall form

Reagents

a. *Stock standard, equivalent to 500 colour units*: Dissolve 1.246 g potassium chloroplatinate, K_2PtCl_6 and 1.00 g crystallised cobaltous chloride, $CoCl_2.6H_2O$ in distilled water with 100 mL conc HCl and dilute to 1000 mL with distilled water.

b. *Working standards*: Prepare working standards according to the following protocol.

Colour units	5	10	15	20	25	30	35
Stock std., mL	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Distilled water mL	49.5	49.0	48.5	48.0	47.5	47.0	46.5

Colour units	40	45	50	55	60	65	70
Stock std., mL	4.0	4.5	5.0	5.5	6.0	6.5	7.0
Distilled water mL	46.0	45.5	45.0	44.5	44.0	43.5	43

Protect the standards against evaporation and contamination when not in use.

Procedure

a. Pour sample in a Nessler tube up to 50 mL mark. Similarly fill three to four tubes with colour standards which appear to correspond to the colour of the sample.

b. Compare colour of the sample with that of the standards by viewing vertically downwards while the tubes are placed on a white surface. Use a colour comparator stand if available.

c. If the sample colour exceeds 70 units, dilute sample with distilled water in known proportions. In case sample contains turbidity report result as apparent colour.

Calculation

a. *For* diluted samples calculate colour units as:

$$\text{Colour Units} = \frac{A \times 50}{B}$$

where:
A = estimated colour of diluted sample
B = mL sample in 50 mL diluted sample

b. Report results in whole numbers recorded as following:

Colour Units	Recorded to Nearest
1-50	1
51-100	5
101-250	10
251-500	20

DO	DISSOLVED OXYGEN
Method:	**WINKLER AZIDE MODIFICATION TITRIMETRIC**
ID: ***1.9***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. *DO sampler,* for collection of undisturbed samples from surface waters.

b. *BOD bottles, 300 mL* , narrow mouth, flared lip, with tapered and pointed ground glass stoppers.

c. *A siphon tube,* for laboratory use.

Reagents

a. *Manganous sulphate solution*. Dissolve 480 g $MnSO_4.4H_2O$, 400 g $MnSO_4.2H_2O$ or 364 g $MnSO_4.H_2O$ in distilled water, filter and dilute to IL.

b. *Alkali-iodide-azide reagent*. Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to IL. Add 10 g NaN_3 dissolved in 40 mL distilled water.

c. *Sulphuric acid, conc*

d. *Starch indicator*. Dissolve 2 g laboratory grade soluble starch and 0.2 g salicylic acid as a preservative, in 100 mL hot distilled water.

e. *Standard sodium thiosulphate titrant, 0.025M (0.025N).* Dissolve 6.205 g $Na_2S_2O_3.5H_2O$ in distilled water. Add 1.5 mL 6*N*NaOH or 0.4 g solid NaOH and dilute to 1000 mL . Standardise with bi-iodate solution.

f. *Standard potassium bi-iodate solution, 0.0021M (0.0126N),* Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 mL .

Standardisation: Take 100 to 150 mL distilled water in an Erlenmeyer flask. Add approximately 2g KI, dissolve. Add 1 mL 6*N* H_2S0_4 or a few drops of conc H_2SO_4 and 20 mL bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulphate titrant to a pale straw colour. Add a few drops of starch indicator. Continue titration to first disappearance of blue colour. Calculate molarity, M of thiosulphate as:

$$M = \frac{20 \times 0.0126}{V}$$

where:
V = mL of thiosulphate used

Procedure

a. Drain any liquid in the flared lip of the BOD bottle containing the sample.

b. Remove stopper and add 1 mL of $MnSO_4$ followed by 1 mL alkali-iodide-azide reagent. Hold the pipette tip just below the liquid surface touching the side of the bottle. Wash the pipette before returning to the reagent bottles.

c. Stopper carefully to exclude air bubbles. Mix by inverting the bottle a few times.

d. Allow the brown manganese hydroxide floc (white floc indicates absence of DO) to settle approximately to half the bottle volume, add 1.0 mL conc H_2SO_4 and re-stopper. Mix by inverting several times until dissolution is complete.

e. Titrate 201 mL with standard $Na_2S_2O_3$ as for standardisation procedure described above.

Calculation

$$\text{mg DO/L} = \frac{V \times M}{0.025}$$

where:

V = mL thiosulphate solution used

M = molarity of thiosulphate titrant

EC	ELECTRICAL CONDUCTIVITY
Method:	**CONDUCTIVITY CELL POTENTIOMETRIC**
ID: ***1.10***	***Version:*** *2*

Apparatus

a. *Conductivity meter* capable of measuring conductivity with an error not exceeding 1% or 0.1mS/m which ever is greater.

b. *Conductivity cell*, Pt electrode type. For new cells not already coated and old cell giving erratic readings platinise according to the following procedure. Clean the cell with chromic - sulphuric acid cleaning mixture. Prepare platinising solution by dissolving 1g chloroplatinic acid, $H_2Pt\ Cl_6.6H_2O$ and 12mg lead acetate in 100 mL distilled water. Immerse electrodes in this solution and connect both to the negative terminal of a 1.5V dry cell battery (in some meters this source is built in). Connect the positive terminal to a platinum wire and dip wire into the solution. Continue electrolysis until both cell electrodes are coated with platinum black.

Reagent

a. *Conductivity water* - use distilled water boiled shortly before use to minimise CO_2 content. Electrical conductivity must be less than 0.01 mS/m (< 0.1 μmho/cm).

b. *Standard potassium chloride solution*, KCl, 0.01*M*, conductivity 141.2 mS/m at 25°C. Dissolve 745.6mg anhydrous KCl (dried 1 hour at 180°C) in conductivity water and dilute to 1000 mL. This reference solution is suitable when the cell has a constant between 1 and 2 per cm.

Procedure

a. Rinse conductivity cell with at least three portions of 0.01M KCl solution. Measure resistance of a fourth portion and note temperature.

b. In case the instrument indicates conductivity directly, and has internal temperature compensation, after rinsing as above, adjust temperature compensation dial to 0.0191/ °C and with the probe in standard KCl solution, adjust meter to read 141.2 mS/m (or 1412 μ mho/cm) continue at step d.

c. *Compute the cell constant*, K_C according to the formula:

$$K_C = \frac{1412}{C_{KCl}} \times [0.0191(t-25)+1]$$

where:
K_c = the cell constant, 1/cm
C_{KCl} = measured conductance, μmho
t = observed temperature of standard KCl solution, °C

The value of temperature correction [0.0191 x (t-25)+1] can be read from Table 1.

d. Rinse cell with one or more portions of sample. The level of sample aliquot must be above the vent holes in the cell and no air bubbles must be allowed inside the cell. Adjust the temperature of sample to about 25°C (outside a temperature range of 20 - 30°C, error increases as the sample temperature increasingly deviates from the reporting temperature of 25°C). Read sample conductivity and note temperature to nearest 0.1°C.

e. Thoroughly rinse the cell in distilled water after measurement, keep it in distilled water when not in use.

Calculation

a. When sample conductivity is measured with instruments having temperature compensation, the readout automatically is corrected to 25°C. If the instrument does not have internal temperature compensation, conductivity at 25°C is:

$$\text{Electrical Conductivity (mS/cm)} = \frac{C_M \times K_C}{0.0191(t-25)+1}$$

where:

K_c = the cell constant, 1/cm

C_M = measured conductance of the sample, mS

t = observed temperature of sample, ^{0}C

The value of temperature correction [0.0191 x (t-25)+1] can be read from Table 1.

b. Record the meter reading, the unit of measurement and the temperature of the sample at the time of reading. Report the electrical conductivity at 25°C. Report conductivity preferably in mS/m, use Table 2 for conversion of units.

T (°C)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
15	0.810	0.812	0.814	0.816	0.818	0.820	0.821	0.823	0.825	0.827
16	0.829	0.831	0.833	0.835	0.837	0.839	0.840	0.842	0.844	0.846
17	0.848	0.850	0.852	0.854	0.856	0.858	0.859	0.861	0.863	0.865
18	0.867	0.869	0.871	0.873	0.875	0.877	0.878	0.880	0.882	0.884
19	0.886	0.888	0.890	0.892	0.894	0.896	0.897	0.899	0.901	0.903
20	0.905	0.907	0.909	0.911	0.913	0.915	0.916	0.918	0.920	0.922
21	0.924	0.926	0.928	0.930	0.932	0.934	0.935	0.937	0.939	0.941
22	0.943	0.945	0.947	0.949	0.951	0.953	0.954	0.956	0.958	0.960
23	0.962	0.964	0.966	0.968	0.970	0.972	0.973	0.975	0.977	0.979
24	0.981	0.983	0.985	0.987	0.989	0.991	0.992	0.994	0.996	0.998
25	1.000	1.002	1.004	1.006	1.008	1.010	1.011	1.013	1.015	1.017
26	1.019	1.021	1.023	1.025	1.027	1.029	1.030	1.032	1.034	1.036
27	1.038	1.040	1.042	1.044	1.046	1.048	1.049	1.051	1.053	1.055
28	1.057	1.059	1.061	1.063	1.065	1.067	1.068	1.070	1.072	1.074
29	1.076	1.078	1.080	1.082	1.084	1.086	1.087	1.089	1.091	1.093
30	1.095	1.097	1.099	1.101	1.103	1.105	1.106	1.108	1.110	1.112
31	1.114	1.116	1.118	1.120	1.122	1.124	1.125	1.127	1.129	1.131
32	1.133	1.135	1.137	1.139	1.141	1.143	1.144	1.146	1.148	1.150
33	1.152	1.154	1.156	1.158	1.160	1.162	1.163	1.165	1.167	1.169
34	1.171	1.173	1.175	1.177	1.179	1.181	1.182	1.184	1.186	1.188
35	1.190	1.192	1.194	1.196	1.198	1.200	1.201	1.203	1.205	1.207

Table 1 Value of [0.0191 x (t-25)+1] for Temperature Correction of EC Measurement

Table 2 Conversion table

Multiply	by	to obtain
μS/m	0.001	mS/m
μS/cm	0.1	mS/m
mS/cm	0.01	mS/m
μmho/cm	0.1	mS/m
mmho/cm	100	mS/m

F	FLUORIDE
Method:	**ION SELECTIVE ELECTRODE METHOD**
ID: ***1.43***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Ion meter

b. Fluoride and reference electrodes

c. Magnetic stirrer with TFE coated stirring bar

Reagents

a. *Stock fluoride solution:* Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000mL; 1.00 mL = 100 μg F^-.

b. *Standard fluoride solution:* Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1 mL = 10 μg F^-.

c. *Fluoride buffer:* Take approximately 500 mL distilled water in a 1-L beaker. Add 57 mL glacial acetic acid, 58 g NaCl and 4 g 1,2 cyclohexylenediaminetetraacetic acid. Place beaker in a cool bath and slowly add 6*N* NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to 1-L volumetric flask and add distilled water to the mark.

Procedure

a. Prepare a series of working standards by diluting 5.0, 10.0 and 20.0 mL of standard solution to 100 mL, corresponding to 0.5, 1.0 and 2 mg F^- /L, respectively.

b. Take between 10 to 25 mL standards and sample in 100 mL beakers. Bring the samples and the standards to the room temperature and add an equal volume of buffer to each beaker. The total volume should be sufficient to immerse the electrode and permit the use of the stirrer.

c. Follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range. Standards already diluted with the buffer may have been supplied with the meter. Avoid stirring before immersing electrodes so as not to entrap air bubbles.

d. If a direct reading instrument is not used, plot on a semilogarithmic graph paper potential measurement of fluoride standards on arithmetic scale vs. fluoride concentration on logarithmic scale.

e. *Important:* Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read fluoride concentration in the sample from the calibration curve or directly from the meter.

F	FLUORIDE
Method:	**SPADNS SPECTROPHOTOMETRIC**
ID: ***1.11***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. *Distillation apparatus*: 1L round bottom long neck, borosilicate glass boiling flask, thermometer adapter, connecting tube and an efficient condenser, with thermometer adapter and a thermometer reading up to 200°C, The apparatus is shown in the Figure. Alternative types of distillation apparatus may be used.

b. *Spectrophotometer for use at 570nm*. It must provide a light path of at least 1 cm or a spectrophotometer with a greenish yellow filter (550 to 580nm).

Reagents

a. *Sulphuric acid*, H_2SO_4, conc., reagent grade

b. *Silver sulphate*, Ag_2SO_4, crystals, reagent grade

c. *Stock fluoride solution*. Dissolve 221.0mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1 mL = 100μg F^-

d. *Standard fluoride solution*. Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1 mL = 10μg F^-

e. *SPADNS solution*: Dissolve 958mg SPADNS, sodium 2 - (parasulphophenylazo)-1,8 - dihydroxy-3,6-naphthalenedisulphonate, in distilled water and dilute to 500 mL; protect from light - stable for 1 year.

f. *Zirconyl-acid reagent*: Dissolve 133mg zirconyl chloride octahydrate, $ZrOCl_2.8H_2O$, in about 25 mL distilled water, add 350 mL conc HCl and dilute to 500 mL.

g. *Mixed acid zirconyl-SPADNS reagent:* Mix equal volumes of SPADNS solution and zirconyl-acid reagent - stable for 2 years.

h. *Reference solution:* Add 10 mL SPADNS solution to 100 mL distilled water. Dilute 7 mL conc HCl to 10 mL with distilled water and add to SPADNS solution - stable for 1 year. Set the instrument to zero with this solution.

i. *Sodium arsenite solution:* Dissolve 5g $NaAsO_2$ and dilute to 1L with distilled water (NOTE: toxic - avoid ingestion).

Procedure

a. Distillation: Distillation is necessary for samples containing high concentration of dissolved solids, see Table. Proceed to step d if distillation is not required. To 400 mL distilled water in the distillation flask, with magnetic stirrer operating, add 200 mL conc. H_2SO_4 and a few glass beads. Connect the apparatus as shown in the figure and heat to

180°C. Prevent overheating by stopping heating when temperature reaches 178°C. Discard distillate.

b. Cool the acid mixture remaining in the flask to 80°C and add 300 mL sample. With stirrer operating, distil until the temperature reaches 180°C (again stop heating at 178°C to prevent overheating), turn off heat; retain the distillate for analysis.

c. Add $AgSO_4$ to the distilling flask at the rate of 5mg/mg Cl^- to avoid Cl^- interference. H_2SO_4 solution in the flask can be used repeatedly until contaminant from samples accumulates to such an extent that recovery is affected. This can be ascertained by distilling a known standard and determining recovery.

d. Standard Curve Preparation: Take the following volumes of standard fluoride solution and dilute to 50 mL with distilled water and note down the temperature:

Standard F^-solution, mL	0	0.1	0.2	0.5	1.0	2.0	3.0	5.0	7.0
μg F^-	0	1.0	2.0	5.0	10.0	20.0	30.0	50.0	70.0

e. Pipette 10.00 mL of mixed acid-zirconyl-SPADNS reagent to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards (at 570nm). Plot a curve of mg F^- versus absorbance. Prepare a new standard curve whenever a fresh reagent or a different standard temperature is used.

f. Sample Pre-treatment: If the sample contains residual chlorine remove it by adding 1 drop (0.05 mL) $NaAsO_2$ solution / 0.1mg residual chlorine and mix.

g. Colour Development: Use a 50 mL sample or a portion made to diluted to 50 mL with distilled water. Adjust sample temperature to that of the standard curve. Set reference point of photometer as above. Add 10.00 mL acid-zirconyl-SPADNS reagent, mix well and read absorbance. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

Calculation

$$\text{mg } F^-/L = \frac{A}{B} \times \frac{I}{R}$$

where:

A = μg F^- reading from the standard curve
B = volume of diluted or undiluted sample taken for colour development, mL
R = when sample is diluted, volume of sample taken for dilution/final volume after dilution.

Notes

Substance	*Concentration, mg/L*	*Type of error*
Alkalinity	5000	-
Aluminum (Al^{3+})	0.1	-
Chloride (Cl^-)	7000	+
Colour & turbidity		Remove or compensate for
Iron	10	-
Phosphate (PO_4^{3-})	16	+
Sulfate (SO_4^{2-})	200	-

Table Concentration of substances causing 0.1mg/L error at 1.0mg F^-/L in SPANDS method. (+) overestimation, (-) underestimation of actual Fluoride concentration.

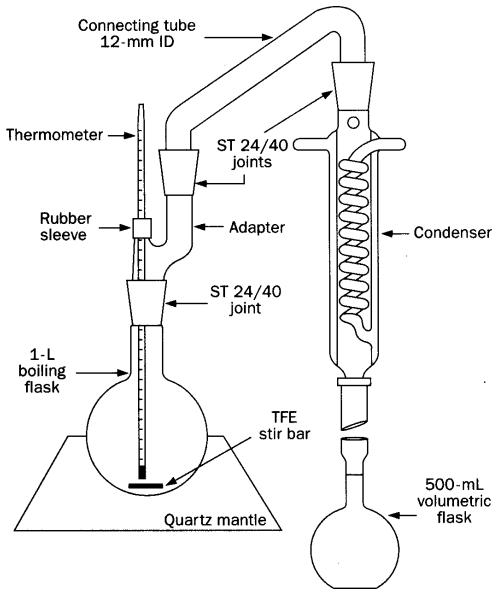


Figure Fluoride Distillation Apparatus

TH	HARDNESS, TOTAL
Method:	**EDTA TITRIMETRIC**
ID: ***1.12***	***Version:*** *2*

Standard Analytical Procedure

Reagents

a. *Buffer solution*[1]: Dissolve 16.9g NH_4Cl in 143 mL conc. NH_4OH. Add 1.25g magnesium salt of ethylenediaminetetraacetate (EDTA) and dilute to 250 mL with distilled water. Store in a plastic bottle stoppered tightly for no longer than one month.

b. *Complexing agent*: Magnesium salt of 1,2 cyclohexanediaminetetraacetic acid. Add 250mg per 100 mL sample only if interfering ions are present and sharp end point is not obtained.

c. *Indicator: Eriochrome Black T sodium salt*. Dissolve 0.5g dye in 100 mL triethanolamine or 2 ethylene glycol monomethyl ether. The salt can also be used in dry powder form by grinding 0.5g dye with 100g NaCl.

d. *Standard EDTA titrant, 0.01M:* Weigh 3.723g di-sodium salt of EDTA, dihydrate, dissolve in distilled water and dilute to 1000 mL. Store in polyethylene bottle.

e. *Standard Calcium Solution*: Weigh 1.000g anhydrous $CaCO_3$ in a 500 mL flask. Add 1 + 1 HCl slowly through a funnel till all $CaCO_3$ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool and add a few drops of methyl red indicator and adjust to the intermediate orange colour by adding 3*N* NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 1mg $CaCO_3$.

Procedure

a. Dilute 25 mL sample to 50 mL with distilled water. Add 1 to 2 mL buffer to give a pH of 10.0 to 10.1. Add 1 to 2 drops of indicator solution and titrate with EDTA titrant to change in colour from reddish tinge to blue. Select a sample volume that requires less then 15 mL EDTA titrant and complete titration within 5 min after buffer addition.

b. Standardise the EDTA titrant against standard calcium solution using the above procedure.

Calculation

$$\text{Total Hardness (EDTA), mg } CaCO_3\text{/L} = \frac{A \times B \times 1000}{\text{mL sample}}$$

where:
A = mL EDTA titrated for sample
B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant

Note

[1)] If the Mg salt of EDTA is unavailable (or too expensive) dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (anlaytical reagent grade) and 780 mg

magnesium sulfate ($MgSO_4.7H_2O$) or 644 mg magnesium chloride ($MgCl_2.6H_2O$) in 50mL 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 distilled water. To attain the highest accuracy, adjust to exact equivalence through appropriate addition of a small amount of EDTA or $MgSO_4$ or $MgCl_2$.

Fe	IRON
Method:	**PHENANTHROLINE SPECTROPHOTOMETRIC**
ID: ***1.13***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer*, for use at 510nm, providing a light path of 1cm or longer

b. *Acid-washed glassware*: use conc. HCl for cleaning all the glassware, rinse with distilled water before use.

c. *Separatory funnels*: 125 mL ground-glass or TFE stopcocks and stoppers

Reagents

a. *Hydrochloric acid*, HCl conc, with less than 0.00005% iron.

b. *Hydroxylamine solution*; dissolve 10g $NH_2OH.HCl$ in 100 mL distilled water.

c. *Ammonium acetate buffer solution*; dissolve 250g $NH_4C_2H_3O_2$ in 150 mL water, add 700 mL glacial acetic acid. Since ammonium acetate may contain a significant amount of iron, prepare new reference standards with each buffer preparation.

d. *Sodium acetate solution;* dissolve 200 g $NaC_2H_3O_2.3H_2O$ in 800 mL water.

e. *Phenanthroline solution*; dissolve 100mg 1,10-Phenanthroline monohydrate, $C_{12}H_8N_2.H_2O$, in 100 mL water by stirring and heating to 80°C, without boiling, discard if darkens or add 2 drops conc. HCl to avoid heating.

f. *Stock iron solution*; slowly add 20 mL conc H_2SO_4 to 50 mL water and dissolve 1.404g ferrous ammonium sulphate, $Fe(NH_4)_2(SO_4)_2.6H_2O$; add 0.1*N* $KMnO_4$ dropwise until a faint pink colour persists, dilute to 1 L with water and mix; 1 mL=200 μgFe.

g. *Standard iron solutions*: (i) Take 50 mL stock iron solution in volumetric flask and dilute to 1 L; 1 mL = 10.0 μgFe; (ii) Take 5 mL stock solution into a 1 L volumetric flask and dilute to the mark with distilled water: 1 mL = 1.0μg Fe.

Procedure

a. Total iron : Take 50 mL of mixed sample into a 125 mL conical flask. If this volume is expected to contain more than 200 μg iron use a smaller portion and dilute to 50 mL. Add 2 mL conc HCl 1 mL NH_2OH. HCl solution, a few glass beads and heat to boiling till the volume is reduced to 15-20 mL, cool, and transfer to a 50 mL volumetric flask. Add 10 mL $NH_4C_2H_3O_2$ buffer solution and 4 mL phenanthroline solution, dilute to the mark with water. Mix and allow 10-15 min. for colour development. Take photometer readings at 510nm.

b. Dissolved iron : Filter sample through a 0.45 μm membrane filter into a vacuum flask containing 1 mL conc.HCl/100 mL sample. Analyse as above and express as total dissolved iron.

c. Ferrous iron : Acidify freshly collected sample with 2 mL conc. HCl/100 mL of sample, withdraw 50 mL portion, add 20 mL phenanthroline solution and 10 mL $NH_4C_2H_3O_2$ solution, mix. Measure the colour after 15 minutes.

d. Calculate ferric iron by subtracting ferrous from total iron.

e. Colour measurement: Prepare a series of standards by accurately pipetting volumes of standard iron solution into 125 mL conical flask, dilute to 50 mL. Follow steps as in *a* and plot a calibration curve. Use weaker standard for measuring 1-10μg iron.

Calculation

Read from the calibration curve and calculate the iron content.

$$\text{mg Fe/L} = \frac{\mu\text{ g Fe (in final volume)}}{\text{mL sample}}$$

Mg	MAGNESIUM
Method:	**CALCULATION FROM TOTAL HARDNESS AND CALCIUM**
ID: ***1.36***	***Version:*** *1*

Standard Analytical Procedure

cedure

Get the values for Total Hardness and Ca Hardness determined by EDTA and calculate Mg

culation

mg Mg/L = (TH as mg $CaCO_3$/L - Calcium Hardness as mg $CaCO_3$/L) x 0.243

where

TH = Total Hardness, mg $CaCO_3$/L

Mn	MANGANESE
Method:	**PERSULPHATE SPECTROPHOTOMETRIC**
ID: ***1.34***	***Version:*** *2*

Standard Analytical Procedure

Apparatus

a. Spectrophotometer, for use at 525 nm, providing a light path of 1 cm or greater.

Reagents

a. *Special reagent:* Dissolve 75 g $HgSO_4$ in 400 mL conc HNO_3 and 200 mL distilled water. Add 200 mL 85% phosphoric acid and 35 mg silver nitrate. Dilute the cooled solution to 1L.

b. *Ammonium persulphate*, $(NH_4)_2S_2O_8$, solid.

c. *Standard manganese solution, 1.00 mL = 50.0 μg Mn:* Dissolve 3.2 g $KMnO_4$ in distilled water and make up to 1 L. Heat for several hours near the boiling point, cool and filter. Standardise against sodium oxalate, as follows:

- Weigh accurately to 0.1 mg, several 100- to 200-mg samples of $Na_2C_2O_4$ and transfer to 400 mL beakers. To each beaker, add 100 mL distilled water and stir to dissolve.
- Add 10 mL 1 + 1 H_2SO_4 and rapidly heat to 90 to 95 ^oC.
- Titrate rapidly with $KMnO_4$ to slight pink end point. Do not let temperature fall below 85^oC. If necessary, warm during titration. Run a blank on distilled water and H_2SO_4.

  Calculate normality:

$$\text{Normality of } KMnO_4 = \frac{\text{g } Na_2C_2O_4}{(A - B) \times 0.06701}$$

  where:

  A = mL titrant for sample

  B = mL titrant for blank

- Average the results of several titrations and calculate volume of this solution necessary to prepare 1 L of standard manganese solution as follows:

$$\text{mL } KMnO_4 = \frac{4.55}{\text{normality of } KMnO_4}$$

- To this volume add 2 to 3 mL conc. H_2SO_4 and $NaHSO_3$ solution drop wise, until the permanganate colour disappears. Boil to remove excess SO_2, cool and dilute to 1000 mL. Dilute this solution further with distilled water to measure small amounts of Mn.

d. *Sodium oxalate:* $Na_2C_2O_4$, primary standard, solid.

e. *Sodium bisulphite:* Dissolve 10 g $NaHSO_3$ in 100mL distilled water.

f. *Hydrogen peroxide*, H_2O_2, 30%.

Procedure

a. Take a suitable volume of sample, containing 0.05 to 2.0 mg Mn, in a 250 mL conical flask. Add 5 mL special reagent and one drop H_2O_2. Concentrate to 90 mL by boiling or dilute to 90 mL

b. Add 1 g $(NH_4)_2S_2O_8$ and boil for 1 min then cool under the tap. Dilute to 100 m L with distilled water.

c. Prepare standards in the range of the sample concentration by treating various amounts of standard Mn solution in the same manner as in *a* and *b* above.

d. Make photometric measurements of standards and sample at 525 nm against a distilled water blank. Use light path of 1 cm for Mn range of 100 – 1500 μg/100mL final reaction volume. Plot standard calibration curve and read Mn concentration in the final 100 mL reaction volume from the standard curve.

Calculation

$$\text{mg Mn/L} = \frac{\mu\text{g Mn/100mL final volume}}{\text{mL sample}}$$

Apparatus

a. *Distillation apparatus* : Borosilicate glass flask 800 mL capacity, attached to a vertical condenser, the outlet tip of which is submerged in the receiving solution.

Reagent

a. *Ammonia free water* : Add 0.1 mL conc. H_2SO_4 to 1 L distilled water, redistill and store in a tightly stoppered glass bottle. Use ammonia free water for preparing reagents, rinsing and dilution.

b. *Borate buffer* : Add 88 mL 0.1*N* NaOH to 500 mL of 9.5 g $Na_2B_4O_7$. IOH_2O/L solution, and dilute to 1 L.

c. *Indicating boric acid solution*: Dissolve 20g H_3BO_3 in water, add 10 mL mixed indicator solution, dilute to 1L. Prepare monthly.

d. *Mixed indicator solution* : Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isoproplyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

e. *Standard sodium carbonate, approximately 0.05N.* Dry 3 to 5g sodium carbonate, Na_2CO_3, at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2g to the nearest mg, dissolve in distilled water and make to 1L.

f. *Standard H_2SO_4, approximately 0.1N*. Dilute 2.8 mL conc. sulphuric acid to 1L. Standardise against 40.00 mL 0.05*N* Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 5. Lift out electrodes, rinse into the same beaker and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker and finish titration to pH 4.3. Calculate normality of sulphuric acid:

$$\text{Normality, N} = \frac{A \times B}{53.00 \times C}$$

where:
A = g Na_2CO_3 weighed into the flask
B = mL Na_2CO_3 solution taken for titration
C = mL acid used

g. In case potentiometric titration is not possible use bromcresol green indicator to complete the titration. The indicator is prepared by dissolving 100g bromcresol green sodium salt in 100 mL distilled water.

h. *Standard sulphuric acid, 0.02N*. Dilute the approximate 0.1*N* solution to 1L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

where:

N = exact normality of the approximate 0.1N solution.

i. *Dechlorinating agent*, dissolve 3.5g sodium thioshuphate, $Na_2S_2O_3.5H_2O$, in water and dilute to 1L, prepare fresh. 1 mL reagent in 500 mL sample will remove 1mg/L chlorine.

Procedure

a. Preparation of equipment: Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6N NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out distillation apparatus.

b. Use 500 mL dechlorinated sample or a known portion diluted to 500 mL. Use the following table to decide on sample volume.

Ammonia Nitrogen in Sample, mg/L	Sample, Volume, mL
0.1-5	500
5-10	250
10-20	100
20-50	50
50-100	25

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

d. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of 50 mL indicting boric acid in a 500 mL Erlenmeyer flask. Collect at least 200 mL distillate. Lower the distillate-receiving flask in the last minute or two to clean condenser and avoid suction of the distillate into the condenser when the heater is turned off.

e. Titrate ammonia in distillate with 0.02 N H_2SO_4 titrant until indicator turns pale lavender.

f. Carry a blank through all steps and apply necessary correction to the results.

Calculation

$$\text{mg } NH_3 - N/L = \frac{(A - B) \times 280}{\text{mL sample}}$$

where :

A = mL $H_2\,SO_4$ titrated for sample

B = mL H_2SO_4 titrated for blank

NH3-N	NITROGEN, AMMONIA
Method:	**PHENATE SPECTROPHOTOMETRIC**
ID: ***1.15***	***Version:*** *1*

Apparatus

a. Spectrophotometer for use at 640nm with a cell of 1cm or longer light path.

Reagents

a. *Phenol solution*: Mix 11.1 mL liquified phenol (≥89%) with 95% V/V ethylalcohol to a final volume of 100 mL. Toxic, avoid personal exposure, discard after a week.

b. *Sodium nitroprusside, 0.5%:* dissolve 0.5g sodium nitroprusside in 100 mL de-ionised water, store in amber bottle, discard after a month.

c. *Alkaline citrate*: Dissolve 200 g trisodium citrate and 10g sodium hydroxide in de-ionised water, dilute to 1L.

d. *Sodium hypochlorite solution, 5%:* Commercial, replace every 2 months.

e. *Oxidizing solution*: Take 100 mL alkaline citrate solution and mix with 25 mL sodium hypochlorite, prepare daily.

f. *Stock ammonium solution*: Weigh 3.819g anhydrous, NH_4Cl, earlier dried at 100^oC and cooled in desiccator, in ammonia free water and dilute to 1L; 1 mL = 1mgN = 1.22 mgNH_3.

g. *Standard ammonium solution:* Prepare dilutions from the stock ammonium solution, in a range appropriate for the concentration of the samples; prepare a calibration curve.

Procedure

a. Take 25 mL sample in a 50 mL conical flask, and add with mixing, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidising solution. Avoid light exposure by suitably covering the flasks at room temperature.

b. Prepare a blank and 2 other ammonia standards in the range, treating in the same way as sample, measure absorbance after 1h at 640nm.

Calculation

Prepare calibration curve by plotting absorbance readings against ammonia concentration of standards, compute sample concentration from the standard curve.

NH3-N	NITROGEN, AMMONIA
Method:	**ION SELECTIVE ELECTRODE METHOD**
ID: ***1.45***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Ion meter

b. Ammonia and reference electrodes

c. Magnetic stirrer with TFE coated stirring bar

Reagents

a. *Ammonia free water:* Add 0.1 mL conc. H_2SO_4 to 1 L distilled water and redistill. Alternatively, prepare de-ionised water from distilled water using a mixed cation and anion exchage resin bed. In case the anion resin releases traces of ammonia, use only a cation exchange resin. Use ammonia free water to prepare all reagents.

b. *Sodium hydroxide, 10N*

c. *NaOH/EDTA solution, 10N:* Dissolve 400g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetra sodium salt, tetrahydrate ($Na_4EDTA.4H_2O$) and stir to dissolve. Cool and dilute to 1000mL.

d. *Stock ammonium chloride solution:* Dissolve 3.819 g anhydrous NH_4Cl (dried at 100 ^oC) in water, dilute to 1000mL; 1 mL = 1mg N.

Procedure

a. Transfer 25 mL of the ammonium chloride stock solution to a 250 mL flask and make up to the mark. Using this diluted standard, transfer 25 mL to another 250 mL flask and make upto the mark.Similarly prepare two more successive dilutions to give serial decimal dilutions of 100, 10, 1.0, and 0.1 mg NH_3-N /L.

b. Place 100 mL of each standard in 150 mL beaker. Immerse electrodes in standard of lowest concentration and mix with magnetic stirrer. Stir at a very low speed to minimise the loss of ammonia under alkaline condition. Add sufficient volume of 10*N* NaOH to raise the pH to 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution. Wait for the reading to stabilise (at least 2 to 3 min) before recording.

c. Repeat *b* above with each of the standards and the samples. Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Maintain the same stirring rate and a temperature of about 25 ^oC throughout calibration and testing procedure.

d. Record the volume of the alkali used if more than 1 mL is used to adjust the pH of any of the samples.

e. Plot on a semilogarithmic graph paper potential measurement, in mV, of the standards in millivolts, on arithmetic scale, vs. mg NH_3-N/L concentration on logarithmic scale. The

calibration curve should be a straight line with a slope of about 59 / decade at 25 ^{o}C. Recalibrate the probes and the instruments several times every day.

f. In case of direct reading ion meters, follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range.

g. *Important:* Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read ammonia nitrogen concentration in the sample from the calibration curve or directly from the meter and correct the reading if more than 1 mL alkali is used as follows:

$$\text{mg}NH_3 - N/L = A \times B \times \frac{100 + D}{100 + C}$$

where:
A = dilution factor for the sample, if any,
B = concentration of NH_3-N, mg/L, from calibration curve
C = volume of alkali added to the standards, mL
D = volume of alkali added to the sample

NO3-N	NITROGEN, NITRATE
Method:	**CALCULATION FROM TON AND NO2-N**
ID: ***1.42***	***Version:*** *2*

Standard Analytical Procedure

cedure

Carry out analysis for TON and NO_2 as descirbed in method 1.41 and 1.17 respectively.

Calculation

$$NO_3^- = TON - NO_2^-$$

where:

TON	=	measured concentration of total oxidised nitrogen ($_3^-$ $+NO_2^-$) by method 1.41, mg N/L
NO_2^-	=	measured concentration of nitrite by method 1.17, mg NO_2^--N/L
NO_3^-	=	concentration $_3^-$, mg NO_3^--N/L

NO3-N	NITROGEN, NITRATE
Method:	**ION SELECTIVE ELECTRODE METHOD**
ID: ***1.44***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Ion meter

b. Nitrate and reference electrodes

c. Magnetic stirrer with TFE coated stirring bar

Reagents

a. *Nitrate free water:* Use double distilled or de-ionised water to prepare all solutions.

b. *Stock nitrate solution:* Dissolve 0.7218 g, previously dried and cooled potassium nitrate (KNO_3) in water and dilute to 1000 mL; 1 mL = 100μg NO_3^- - N.

c. *Standard nitrate solutions:* Dilute 1.0, 10, and 50 mL stock nitrate solution to 100 mL to obtain standards of 1.0, 10 and 50 mg NO_3^- - N/L, respectively.

d. *Buffer solution:* Dissolve 17.32 g $Al_2(SO_4)_3.18H_2O$, 3.43 g Ag_2SO_4, 1.28 g H_3BO_3, and 2.52 g sulfamic acid (H_2NSO_3H), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10 *N* NaOH. Dilute to 1000 mL and store in a dark glass bottle.

Procedure

a. Transfer 10 mL of 1.0 mg NO_3^- - N/L standard to a 50 mL beaker, add 10 mL buffer and stir with magnetic stirrer. Stop stirring after mixing and immerse electrodes. Start stirring again.

b. Take millivolt reading when stable (after about 1 min). Repeat with 10 and 50 mg NO_3-N/L standards.

c. Plot on a semilogarithmic graph paper potential measurement of the standards in mV, on arithmetic scale, vs. NO_3^- - N concentration on logarithmic scale. The calibration curve should be a straight line with a slope of +57 ±3/ decade at 25 °C. Recalibrate the probes and the instruments several times every day using the 10 mg NO_3^--N/L standard.

d. Transfer 10 mL sample to a 50 mL beaker, add 10 mL buffer and stir with magnetic stirrer. Stop stirring after mixing and immerse electrodes. Start stirring again. Take millivolt reading when stable (after about 1 min).

e. In case of direct reading ion meters, follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range. Standards already diluted with the buffer may have been supplied with the meter.

f. *Important:* Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read nitrate nitrogen concentration in the sample from the calibration curve or directly from the meter.

NO3-N	NITROGEN, NITRATE
Method:	**UV SPECTROPHOTOMETRIC**
ID: ***1.16***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer*, for use at 220nm and 275nm with matched Silica cells of 1 cm or longer light path.

Reagents

a. *Nitrate free water*: Use re-distilled or de-ionised water to prepare all solutions.

b. *Stock Nitrate solution*: Dissolve 0.7218g KNO_3, earlier dried in hot air oven at 105^oC overnight and cooled in desiccator, in distilled water and dilute to 1L. Preserve with 2 mL of $CHCl_3$/L; 1 mL = 100 μg NO_3^--N, stable for 6 months.

c. *Standard Nitrate Solution*: Dilute 100 mL of stock solution to 1000 mL with water, preserve with 2 mL $CHCl_3$/l; 1 mL = 10 μg NO_3^--N, stable for 6 months.

d. *Hydrochloric acid solution*, *HCl, 1N*: Cautiously add 83 mL conc. HCl to about 850 mL of distilled water while mixing, cool and dilute to 1L.

Procedure

a. Treatment of sample: Add 1 mL HCl to 50 mL clear/filtered sample, mix.

b. Preparation of standard curve: Prepare calibration standards in the range of 0-7 mg NO_3^--N/L, by diluting to 50 mL the following volumes of standard solutions, add 1 mL of HCl and mix.

Nitrate Standard solution, mL	*1*	*2*	*4*	*7*	*10*	*15*	*20*	*25*	*30*	*35*
NO_3^--N, mg/L	*0.2*	*0.4*	*0.8*	*1.4*	*2.0*	*3.0*	*4.0*	*5.0*	*6.0*	*7.0*

c. Spectrophotometric measurements: Read absorbance or transmittance against re-distilled water set at zero absorbance or 100 % transmittance. Use a wavelength of 220 □m to obtain NO_3^- reading and a wavelength of 275nm to determine interference due to dissolved organic matter

d. *If reading at 275 □m is more than10% of the reading at 220nm, do not use this method (use method 1.42 instead).*

Calculation

For sample and standards, subtract 2 times the absorbance reading at 275nm, from the reading at 220nm to obtain absorbance due to NO_3^-. Prepare a standard curve by plotting absorbance due to NO_3^- against NO_3^--N concentration of standards. Obtain sample concentrations directly from standard curve, by using corrected sample absorbances.

NO2-N	NITROGEN, NITRITE
Method:	**SULPHANILAMIDE SPECTROPHOTOMETRIC**
ID: ***1.17***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer for use at 543nm* or filter photometer with green filter, maximum transmittance near 540nm, providing 1 cm light path or longer.

Reagents

a. *Colour reagent*: To 800 mL water add 100 mL 85% phosphoric acid and 10g sulphanilamide. After dissolving add 1g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1L with water. Solution is stable for one month when stored in dark bottle in refrigerator.

b. *Sodium oxalate*, 0.025M (0.05N): Dissolve 3.350g $Na_2C_2O_4$ primary standard grade, in water and dilute to 1000 mL.

c. *Stock nitrite*: Dissolve 1.232g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 μgN. Preserve with 1 mL $CHCl_3$. Standardise by pipetting, in order, 50 mL 0.01M $KMnO_4$, 5 mL conc H_2SO_4 and 50.00 mL stock NO_2^- solution in to a glass stoppered flask. Shake gently and warm to 70-80°C. Discharge permanganate colour by adding 10 mL portions of 0.025M sodium oxalate. Titrate excess oxalate with 0.01M (0.05N) $KMnO_4$ to faint pink end point. Calculate nitrite content of stock solution:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:
A = mg NO_2^- - N/mL in stock solution
B = mL total $KMnO_4$ used
C = normality of $KMnO_4$
D = total mL oxalate added
E = normality of oxalate
F = mL stock nitrite taken for titration

d. *Intermediate nitrite solution*: Calculate the volume, G, of stock nitrite solution required for the intermediate nitrite solution from G = 12.5/A. Dilute the volume G to 250 mL with water; 1 mL = 50.0μg NO_2^--N. Prepare daily.

e. *Standard nitrite solution*: Dilute 10 mL intermediate NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500μg NO_2^--N. Prepare daily.

f. *Standard potassium permanganate titrant,* 0.01M (0.05*N*): Dissolve 1.6g $KMnO_4$ in 1L distilled water. Allow ageing for 1 week then decant supernatant. Standardise this solution frequently as follows:

Weigh to nearest 0.1mg several 100 to 200mg samples of anhydrous sodium oxalate in beakers. To each beaker add 100 mL distilled water, 10 mL 1 + 1 H_2SO_4 and heat rapidly to 90 to 95°C. Titrate with permanganate solution to a slight pink end point that persists to at least 1 min. Do not allow temperature to fall below 85°C. Run a blank on distilled water + H_2SO_4.

$$\text{normality } KMnO_4 = \frac{g\, Na_2C_2O_4}{(A - B) \times 0.33505}$$

where:

A = mL titrant for sample
B = mL titrant for blank

Average the result of several titrations.

Procedure

a. Add 2 mL colour reagent to 50 mL sample, or to a portion diluted to 50 mL, and mix.
b. Measure absorbance at 543nm. Wait between 10 min and 2h after addition of colour reagent before measurement
c. Prepare standard curve by diluting 1, 2, 3, 4 and 5 mL of standard nitrite solution to 100 mL to give 5, 10, 15, 20 and 25 μg/L concentration, respectively.

Calculation

Compute sample concentration directly from the curve, taking in consideration dilution of the sample if applicable.

Org-N	NITROGEN, ORGANIC
Method:	**KJELDAHL TITRIMETRIC**
ID: ***1.18***	***Version:*** *2*

Apparatus

a. *Digestion apparatus*, a heating device to provide temperature range of 375°C to 385 °C for effective digestion, adjusted to boil 250 mL of water in 800 mL total capacity Kjeldahl flask in about 5 min.

b. *Distillation apparatus*, a borosilicate glass flask of 800 mL capacity attached to a vertical condenser, the outlet tip of the condenser is submerged in the receiving acid solution.

Reagents

a. *Ammonia free water*. Add 0.1 mL conc. H_2SO_4 to 1L distilled water, redistill and store in a tightly stopped glass container, prepare fresh. Use ammonia free water for preparing reagents, rinsing and dilution.

b. *Borate Buffer solution*, add 88 mL 0.1 *N* NaOH solution to 500 mL of approximately 0.025 M sodium tetraborate, (9.5g $Na_2B_4O_7.10H_2O$/L) and dilute to 1L.

c. *NaOH*, 6N

d. *Dechlorinating agent*, dissolve 3.5g sodium thioshuphate, $Na_2S_2O_3.5H_2O$, in water and dilute to 1L, prepare fresh. 1 mL reagent in 500 mL sample will remove 1mg/L chlorine.

e. *Neutralising agents:* NaOH, 1*N, H_2SO_4, 1N*

f. *Mixed indicator solution*: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine the solutions and prepare every month.

g. *Indicating boric acid solution*. Dissolve 20g H_3BO_3 in water, add 10 mL mixed indicator solution and dilute to 1L, prepare every month.

h. *Sulphu*ric acid, 0.04N: Dilute 1 mL conc H_2SO_4 to 1L.

i. Standard sodium carbonate, approximately 0.05N. Dry 3 to 5g sodium carbonate, Na_2CO_3, at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2g to the nearest mg, dissolve in distilled water and make to 1L.

j. *Standard H_2SO_4, approximately 0.1N*. Dilute 2.8 mL conc. s*ulphuric acid to* 1L. Standardise against 40.00 mL 0.05*N* Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 5. Lift out electrodes, rinse into the same beaker and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker and finish titration to pH 4.3. Calculate normality of sulphuric acid:

$$\text{Normality, N} = \frac{\text{A} \times \text{B}}{53.00 \times \text{C}}$$

where:
A = g Na_2CO_3 weighed into the flask
B = mL Na_2CO_3 solution taken for titration
C = mL acid used

k. In case potentiometric titration is not possible use bromcresol green indicator to complete the titration. The indicator is prepared by dissolving 100g bromcresol green sodium salt in 100 mL distilled water.

l. *Standard sulphuric acid, 0.02N*. Dilute the approximate 0.1*N* solution to 1L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

where:
N = exact normality of the approximate 0.1*N* solution.

m. *Digestion reagent:* Dissolve 134g K_2SO_4 and 7.3g $CuSO_4$ in about 800 mL water. Slowly add 134 mL conc. H_2SO_4 with mixing, cool to room temperature, dilute to 1L with water.

n. *Sodium hydroxide thiosulphate reagent*. Dissolve 500g NaOH and 25g $Na_2S_2O_3.5H_2O$ in water and dilute to 1L.

o. *Sodium hydroxide, 6N*

Procedure

a. Select sample size from the table:

Organic Nitrogen in sample, mg/l	0-1	1-10	10-20	20-50	50-100
Sample size, mL	500	250	100	50	25

If necessary, dilute to 300 mL, neutralise to pH 7 and dechlorinate by using 1 mL reagent to remove 1mg/L residual chlorine in 500 mL sample.

b. Ammonia removal: Add 25 mL borate buffer and 6*N* NaOH until pH9.5 is reached. Add a few beads and boil off 300 mL. If desired distill this fraction and determine ammonia nitrogen.

c. Alternately, if ammonia has been determined by distillation method, use residue in the distilling flask for organic nitrogen determination.

d. Cool, add carefully 50 mL digestion reagent to distillation flask. Add a few glass beads, mix, heat with suitable ejection equipment to remove acid fumes, under a fume cupboard. Boil, until the volume is reduced to 25-50 mL, and copious white fumes, observed and the turbid sample becomes transparent and pale green. After digestion, cool, dilute to 300 mL with water and mix. Carefully add 50 mL sodium hydroxide-thiosulphate reagent to form an alkaline layer at flask bottom. Connect flask to a distillation apparatus. The pH of the solution should exceed 11.0

e. Distil and collect 200 mL distillate in 50 mL indicator boric acid as absorbent solution, keeping the condenser tip well below the absorbent solution surface. Lower the collected distillate free of contact with condenser tip and continue distillation for further 2 min. to clean the condenser.

f. Titrate the distillate with standard 0.02*N* H_2SO_4 titrant until indicator turns pale lavender.

g. Carry a blank through all steps of the procedure.

Calculation

$$\text{mgN/L} = \frac{(\text{A} - \text{B}) \times 280}{\text{mL sample}}$$

where:
A = mL of H_2SO_4 titrated for sample
B = mL of H_2SO_4 titrated for blank

TON	NITROGEN, TOTAL OXIDISED (NO2+NO3)
Method:	**CD REDUCTION + SPECTROPHOTOMETRIC NO2**
ID: ***1.41***	***Version:*** *1*

Apparatus

a. *Reduction colum*n: Columns can be constructed by modification of 100mL volumetric pipette (see figure 1). Metering valve is recommended to control flow rate.

b. *Spectrophotometer for use at 543nm* or filter photometer with green filter, maximum transmittance near 540nm, providing 1 cm light path or longer.

Reagents

a. *Nitrate free water*. Use as reagent blank. The NO_3-N concentration should not exceed 0.01 mg/L. Use nitrate free water for all solutions an dilutions.

b. *Copper-cadmium granules*: Wash 25g 20- to 100-mesh Cd granules with *6N* HCl and rinse with water. Swirl granules with 100 mL 2% $CuSO_4$ till blue colour partially fades. Decant and repeat with fresh Cu solution until brown colloidal precipitate is seen. Rinse with nitrate free water to remove colloidal Cu precipitate.

c. *Ammonium chloride-EDTA solution:* Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust pH to 8.5 with conc. NH_4OH and dilute to 1 L.

d. *Dilute Ammonium chloride-EDTA solution:* Dilute 300 mL Ammonium chloride-EDTA solution to 500 mL with water.

e. *Hydrochloric acid, HCl, 6N.*

f. *Copper sulphate solution, 2%:* Dissolve 20 g $CuSO_4.5H_2O$ in 500 mL water and dilute to 1L

g. *Colour reagent*: To 800 mL water add 100 mL 85% phosphoric acid and 10g sulphanilamide. After dissolving add 1g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1L with water. Solution is stable for one month when stored in dark bottle in refrigerator.

h. *Stock nitrite*: Dissolve 1.232g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 μgN. Preserve with 1 mL $CHCl_3$. Standardise by pipetting, in order, 50 mL 0.01M $KMnO_4$, 5 mL conc. H_2SO_4 and 50.00 mL stock NO_2^- solution in to a glass stoppered flask. Shake gently and warm to 70-80°C. Discharge permanganate colour by adding 10 mL portions of 0.025M sodium oxalate. Titrate excess oxalate with 0.01M (0.05N) $KMnO_4$ to faint pink end point. Calculate nitrite content of stock solution:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

A = mg NO_2^- - N/mL in stock solution

B = mL total $KMnO_4$ used
C = normality of $KMnO_4$
D = total mL oxalate added
E = normality of oxalate
F = mL stock nitrite taken for titration

i. *Intermediate nitrite solution*: Calculate the volume, G, of stock nitrite solution required for the intermediate nitrite solution from G = 12.5/A. Dilute the volume G to 250 mL with water; 1 mL = 50.0μg NO_2^--N. Prepare daily.

j. *Stock Nitrate solution*: Dissolve 0.7218g KNO_3, earlier dried in hot air oven at 105°C overnight and cooled in desiccator, in distilled water and dilute to 1L. Preserve with 2 mL of $CHCl_3$/L; 1 mL = 100 μg NO_3^--N, stable for 6 months.

k. *Standard Nitrate Solution*: Dilute 100 mL of stock solution to 1000 mL with water, preserve with 2 mL $CHCl_3$/l; 1 mL = 10 μg NO_3^--N, stable for 6 months.

l. *Working Nitrite solution:* Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite free water; 1mL = 5 □g NO_2^--N.

cedure

a. *Prepare reduction columns*: Insert glass wool plug into bottom of column and fill with water. Add sufficient Cd granules to obtain a length of 18.5 cm. Prevent air entrapment by keeping water level abouve granules. Wash the column with 200 mL NH_4Cl-EDTA solution. Activate the column by flushing at least 100 mL of a mixture of 25% 1.0 mg NO_3^--N/L standard an 75% NH_4Cl-EDTA solution. Flush-rate should be between 7 and 10 mL/min.

b. *Pre-treatment of the sample*: Remove turbidity, if any, by filtering through 0.45 □m membrane fillter. Adust pH between 7 and 9 if needed. Use pH-meter, dilute HCl and NaOH

c. *Sample reduction:* Obtain a 25 mL sample or a portion diluted upto less than 4 mg/L NO_3^--N/L) and mix with 75 mL NH_4Cl-EDTA solution. Flush mixture over the column at a rate of 7 to 10 mL/min. Discard first 25 mL and collect rest in original sample flask. Analyse within 15 minutes. Washing column inbetween different samples is not needed. If colums are not used for more than several hours flush with 50 mL NH_4Cl-EDTA solution. Never let column dry, granules should be immersed.

d. *Colour development*: As soon as possible but at least within 15 minutes after reduction add 2 mL colour reagent to 50 mL sample and mix.

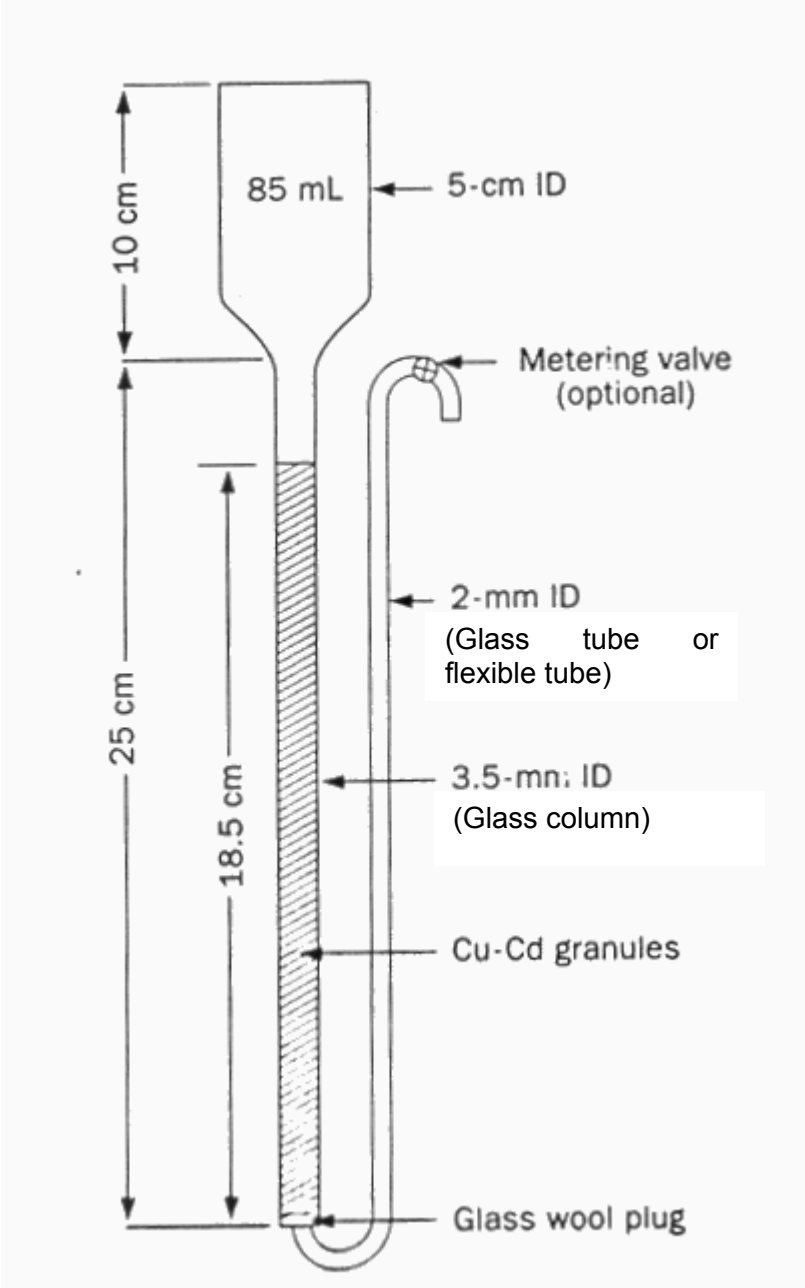
e. *Measurement of absorbance at 543nm*. Wait between 10 min and 2h after addition of colour reagent before measurement. Measure against a distilled nitrate free water reagent blank. If NO_3^- concentration exceeds highest standard (1 mg/L) use remaining portion of reduced sample to make appropriate dilution and develop colour again

f. *Standards:* Use the intermediate NO_3^--N solution to prepare standards in the range of 0.05 to 1.0 mg NO_3^--N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0 and 10.0 mL. Carry out reduction of standards exactly as for samples.

g. *Column efficiency check*: Using an appropriate dilution of the working nitrite (NO_2^-) solution, compare to a reduced nitrate (NO_3^-) standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granueles as described abouve when reduction efficiency falls below 75%.

culation

Obtain a standard curve by plotting the absorbance of at least 5 standards against their NO_3^--*N* concentration. Read sample concentration directly from standard curve. Account for dilutions if any and report as milligrams oxidised N per litre (TON or NO_3^--N plus NO_2^--N).



OD	ODOUR
Method:	**QUALITATIVE HUMAN RECEPTOR**
ID: ***1.19***	***Version:*** *1*

Standard Analytical Procedure

Procedure

a. As soon as possible after collection of sample, fill a cleaned odourless bottle half - full of sample, insert stopper, shake vigorously for 2 to 3 seconds and then quickly observe the odour. The sample should be at ambient temperature.

b. Report the odour as: odour free, rotten egg, burnt sugar, soapy, fishy, septic, aromatic, chlorinous, alcoholic odour or any other specific odour. In case it is not possible to specify the exact nature of odour, report as agreeable or disagreeable.

pH	PH
Method:	**POTENTIOMETRIC**
ID: ***1.21***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *pH meter* with temperature compensating device, accurate and reproducible to 0.1 pH unit with a range of 0 to 14.

b. *Reference electrode* preferably with quartz liquid junction. Follow manufacturer's instructions on use and care of the reference electrode. Refill non-sealed electrodes with correct electrolyte to proper level and make sure junction is properly wetted.

c. *Glass electrode*. Follow manufacturer's instructions on use and care of electrode.

Reagents

a. *Potassium hydrogen phthalate buffer, 0.05M*, pH 4.00. Dissolve 10.12 g $KHC_8H_4O_4$ (potassium hydrogen phthalate) in 1000 mL freshly boiled and cooled distilled water

b. *0.025M Potassium dihydrogen phosphate + 0.025M disodium hydrogen phosphate buffer, pH 6.86*. Dissolve 3.387 g KH_2PO_4 + 3.533 g Na_2HPO_4 in 1000 mL freshly boiled and cooled distilled water

c. *0.01M sodium borate decahydrate (borax buffer), pH = 9.18*. Dissolve 3.80 g $Na_2B_4O_7.10H_2O$ in 1000 mL freshly boiled and cooled distilled water.

d. Store buffer solutions in polyethylene bottles. Replace buffer solutions every 4 weeks.

Procedure

a. Remove electrodes from storage solution, rinse, blot dry with soft tissue, place in initial buffer solution and standardise pH meter according to manufacturer's instructions.
b. Remove electrodes from the first buffer, rinse thoroughly with distilled water, blot dry and immerse in second buffer preferably of pH within 2 pH units of the pH of the sample. Read pH, which should be within 0.1 unit of the pH of the second buffer.
c. Determine pH of the sample using the same procedure as in (b) after establishing equilibrium between electrodes and sample. For buffered samples this can be done by dipping the electrode into a portion of the sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.
d. With dilute poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of the sample. Take a fresh sample to measure pH.
e. Stir the sample gently while measuring pH to insure homogeneity.

o-PO4-P	PHOSPHORUS, ORTHO PHOSPHATE
Method:	**ASCORBIC ACID SPECTROPHOTOMETRIC**
ID: ***1.20***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer with infrared phototube* for use at 880nm or filter photometer, equipped with a red filter.

b. *Acid washed glassware*, use dilute HCl and rinse with distilled water.

Reagents

a. *Sulphuric acid*, H_2SO_4, 5N: Dilute 70 mL conc. H_2SO_4 to 500 mL with distilled water.

b. *Potassium antimonyl tartrate solution*: Dissolve 1.3715g $K(SbO)C_4H_4O_6.1/2\ H_2O$ in 400 mL distilled water and dilute to 500 mL, store in glass-stoppered bottle.

c. *Ammonium molybdate solution*: Dissolve 20g $(NH_4)6\ Mo_7O_{24}.4H_2O$ in 500 mL distilled water, store in a glass stoppered bottle.

d. *Ascorbic acid, 0.1M:* Dissolve 1.76g ascorbic acid in 100 mL distilled water, keep at 4°C, use within a week.

e. *Combined reagents*: Mix 50 mL 5N, H_2SO_4, 5 mL potassium antimonyl tartrate, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution, in the order given and at room temperature. Stable for 4 hours.

f. *Stock phosphate solution*, Dissolve 219.5mg anhydrous KH_2PO_4 in distilled water and dilute to 1 L; 1 mL = 50μg PO_4^{3-} - P.

g. *Standard phosphate solution*: Dilute 50 mL stock solution to 1L with distilled water; 1 mL = 2.5μg P.

Procedure

a. Treatment of sample: Take 50 mL sample into a 125 mL conical flask, add 1 drop of phenolphthalein indicator. Discharge any red colour by adding 5*N* H_2SO_4. Add 8 mL combined reagent and mix.

b. Wait for 10 minutes, but no more than 30 minutes and measure absorbance of each sample at 880nm. Use reagent blank as reference.

c. Correction for turbid or coloured samples. Prepare a sample blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from sample absorbance reading.

d. Preparation of calibration curve: Prepare calibration from a series of standards between 0.15-1.30 mgP/L range (for a 1 cm light path). Use distilled water blank with the combined reagent. Plot a graph with absorbance versus phosphate concentration to give a straight line. Test at least one phosphate standard with each set of samples.

Calculation

$$o\text{–}PO_4 \text{ as mg P/L} = \frac{\text{mg P}_{\text{from the calibration curve}} \times 1000}{\text{mL sample}}$$

P-Tot	PHOSPHORUS, TOTAL
Method:	**DIGESTION AND ASCORBIC ACID SPECTROPHOTOMETRIC**
ID: ***1.39***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Hot plate.

b. *Spectrophotometer with infrared phototube* for use at 880nm or filter photometer, equipped with a red filter.

c. *Acid washed glassware*, use dilute HCl and rinse with distilled water.

Reagents

a. Phenolphthalein indicator aqueous solution

b. Sulphuric acid ,H_2SO_4 10N: Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L.

c. Persulphate: $(NH_4)_2S_2O_8$ or $K_2S_2O_8$, solid

d. Sulphuric acid, H_2SO_4, 5N: Dilute 70 mL conc. H_2SO_4 to 500 mL with distilled water.

e. *Potassium antimonyl tartrate solution*: Dissolve 1.3715g $K(SbO)C_4H_4O_6.1/2\ H_2O$ in 400 mL distilled water and dilute to 500 mL, store in glass-stoppered bottle.

f. *Ammonium molybdate solution*: Dissolve 20g $(NH_4)_6\ Mo_7O_{24}.4H_2O$ in 500 mL distilled water, store in a glass stoppered bottle.

g. *Ascorbic acid, 0.1M:* Dissolve 1.76g ascorbic acid in 100 mL distilled water, keep at 4^{o}C, use within a week.

h. *Combined reagents*: Mix 50 mL 5N, H_2SO_4, 5 mL potassium antimonyl tartrate, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution, in the order given and at room temperature. Stable for 4 hours.

i. *Stock phosphate solution*, Dissolve 219.5mg anhydrous KH_2PO_4 in distilled water and dilute to 1 L; 1 mL = 50μg PO_4^{3-} - P.

j. *Standard phosphate solution*: Dilute 50 mL stock solution to 1L with distilled water; 1 mL = 2.5μg P.

Procedure

a. To 50 mL portion of thoroughly mixed sample add one drop phenolphthalein indicator solution. If a red colour develops, add 10N H_2SO_4 to just discharge colour. Then add 1 mL 10N H_2SO_4 and either 0.4 g $(NH_4)_2S_2O_8$ or 0.5 g $K_2S_2O_8$.

b. Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached.

c. Cool, dilute to 30 mL with distilled water, add one drop phenolphthalein indicator solution and neutralize to a faint pink colour with NaOH and make up to 100 mL with distilled water. Do not filter if any precipitate is formed at this stage. It will redissolve under acid conditions of the colourometric test.

d. Take 50 mL of the digested sample into a 125 mL conical flask, add 1 drop of phenolphthalein indicator. Discharge any red colour by adding 5*N* H_2SO_4. Add 8 mL combined reagent and mix.

e. Wait for 10 minutes, but no more than 30 minutes and measure absorbance of each sample at 880nm. Use reagent blank as reference.

f. Correction for turbid or coloured samples. Prepare a sample blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from sample absorbance reading.

g. Preparation of calibration curve: Prepare calibration from a series of standards between 0.15-1.30 mgP/L range (for a 1 cm light path) by first carrying the standards through identical persulphate digestion process. Use distilled water blank with the combined reagent. Plot a graph with absorbance versus phosphate concentration to give a straight line. Test at least one phosphate standard with each set of samples.

culation

$$\text{Total P as mg P/L} = \frac{\text{mg P}_{\text{from the calibration curve}} \times 1000}{\text{mL sample}}$$

K	POTASSIUM
Method:	**FLAME EMISSION PHOTOMETRIC**
ID: ***1.35***	***Version:*** *1*

Standard Analytical Procedure

paratus

a. *Flame photometer*, direct reading type.

b. *Glassware,* rinse with 1 + 15 HNO_3, followed by de-ionised distilled water.

c. *Plastic bottles*, to store all solutions

ngents

a. *Stock potassium solution*, weigh 1.907g KCl, dried at 110°C and cooled in desiccator, transfer to 1L volumetric flask and make to 1L with water; 1mL = 1.00mg K.

b. *Intermediate potassium solution*, dilute 10mL stock potassium solution with water to 100mL; 1mL = 0.1mg K, prepare calibration curve in the range of 1 to 10mg/L

c. *Standard potassium solution*: Dilute 10mL intermediate solution with water to 100mL, 1mL = 10μg K, prepare calibration curve in the range of 0.1 to 1mg/L.

Procedure

a. Follow instructions of flame photometer manufacturer for selecting proper photocell, wavelength, slit width adjustments, fuel gas and air pressure, steps for warm up, correcting for interference and flame background, rinsing of burner, sample ignition and emission intensity measurements.

b. Prepare a blank and potassium calibration standards, in any of the applicable ranges, 0-100, 0-10, or 0-1 mg K/L. Measure emission at 766.5 nm and prepare calibration curve. Determine potassiumium concentration of the sample, or diluted sample, from the curve.

culation

$$\text{mg K/L} = \text{mg K/L}_{\text{ from the calibration cuve}} \times \text{Dilution}$$

where:

$$\text{Dilution} = \frac{\text{mL sample} + \text{mL distilled water}}{\text{mL sample}}$$

SiO2	SILICATE
Method:	**AMMONIUM MOLYBDATE SPECTROPHOTOMETRIC**
ID: ***1.38***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Spectrophotometer*, for use at 815 nm, having 1 cm light path.

Reagents

a. Store all reagents in plastic containers.

b. *Sulphuric acid,* H_2SO_4, 1N.

c. *Hydrochloric acid,* HCl, 1 + 1

d. *Ammonium molybdate reagent:* Dissolve 10 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in distilled water, with stirring and gentle warming, and dilute to 100 mL. Filter if necessary. Adjust pH between 7 and 8 with silica free NH_4OH or NaOH and store in polyethylene bottle to stabilise.

e. *Oxalic acid solution:* Dissolve 7.5 g $H_2C_2O_4.H_2O$ in distilled water and dilute to 100 mL.

f. *Stock silica solution:* Dissolve 313.0 mg sodium hexafluorosilicate,Na_2SiF_6, in 1000 mL distilled water; 1 ml = 0.1 mg SiO_2.

g. *Silica standard working solution:* Dilute 100 mL stock solution to 1000mL; 1mL = 10 μg SiO_2.

h. *Reducing agent:* Dissolve 500 mg 1-amino-2-naphthol-4-sulphonic acid and 1 g Na_2SO_3 in 50 mL distilled water, with gentle warming if necessary; add this to a solution of 30 g $NaHSO_3$ in 150 mL distilled water. Filter into a plastic bottle. Discard when the solution becomes dark. Prolong reagent life by storing a refrigerator away from light.

cedure

a. To 50 mL sample, containing between 20 and 100 μg silica, add in rapid succession 1.0 mL (1 + 1) HCl and 2 mL ammonium molybdate reagent. Mix thoroughly and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix. Measuring time from the moment of adding oxalic acid, wait at least 2 min but not more than 15 min, add 2 mL reducing agent and mix thoroughly.

b. Read absorbance at 815 nm after 5 min, adjusting the instrument to zero absorbance using distilled water blank.

c. Dilute 2.0, 4.0, 6.0, 8.0 and 10.0 mL silica working standard solution to 50 mL volumes and proceed as in *a* and *b* above to prepare a calibration curve.

culation

Read silica content of sample from the calibration curve.

Note

Standard Methods recommends use of sodium metasilicate nonahydrate, $Na_2SiO_3.9H_2O$, for preparation of standards and its standardisation by gravimetric method.
The method measures molybdate reactive silica. To determine molybdate unreactive silica a digestion step is necessary.

Na	SODIUM
Method:	**FLAME EMISSION PHOTOMETRIC**
ID: ***1.22***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Flame photometer*, direct reading type.

b. *Glassware,* rinse with 1 + 15 HNO_3, followed by de-ionised distilled water.

c. *Plastic bottles*, to store all solutions.

Reagents

a. *Stock sodium solution*, weigh 2.542g NaCl, dried at 140^{o}C and cooled in desiccator, transfer to 1L volumetric flask and make to 1L with water; 1 mL = 1.00mg Na.

b. *Intermediate sodium solution*, dilute 10 mL stock sodium solution with water to 100 mL; 1 mL = 0.1mg Na, prepare calibration curve in the range of 1 to 10mg/L

c. *Standard sodium solution*: Dilute 10 mL intermediate solution with water to 100 mL, 1 mL = 10μg Na, prepare calibration curve in the range of 0.1 to 1mg/L.

Procedure

a. Follow instructions of flame photometer manufacturer for selecting proper photocell, wavelength, slit width adjustments, fuel gas and air pressure, steps for warm up, correcting for interference and flame background, rinsing of burner, sample ignition and emission intensity measurements.

b. Prepare a blank and sodium calibration standards, in any of the applicable ranges, 0-100, 0-10, or 0-1 mg Na/L. Set instrument zero with standard containing no sodium. Measure emission at 589nm and prepare calibration curve. Determine sodium concentration of the sample, or diluted sample, from the curve.

Calculation

$$\text{mgNa/L} = \text{mgNa/L}_{\text{ from the calibration cuve}} \times \text{Dilution}$$

where:

$$\text{Dilution} = \frac{\text{mL sample} + \text{mL distilled water}}{\text{mL sample}}$$

TSS	SOLIDS, TOTAL SUSPENDED
Method:	**GRAVIMETRIC AFTER FILTRATION**
ID: ***1.24***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Glass-fibre filter disk, Whatman grade 934 AH, Gelman type A/E, Millipore type AP4O or equivalent, diameter 2.2 to 12.5 cm.

b. Filtration apparatus, Membrane filter funnel or Gooch crucible with adapter and suction flask of sufficient capacity for sample size selected

c. Drying oven, 104 $\pm$ 1 ^{0}C

d. Analytical balance capable of weighing up to 0.1 mg.

e. Aluminium weighing dishes.

Procedure

a. Wash filter paper by putting it on filtration assembly and filtering 3 successive 20 mL portions of distilled water.

b. Place filter in an aluminium dish and dry in oven at 104 $\pm$ 1^{o}C for 1 h. If a Gooch crucible is used, dry filter and crucible combination together. Cool in desiccator to balance temperature and weigh.

c. Assemble filtration apparatus with the washed, dried and weighed filter paper. Wet filter paper with a small amount of distilled water to seat it.

d. Stir sample with a magnetic stirrer and while stirring pipette a measured volume on to the filter using a wide bore pipette. Choose sample volume to yield between 10 and 200 mg dried solids.

e. Wash with 3 successive 10 mL volumes of distilled water. Continue suction for about 3 min after filtration is complete.

f. Carefully remove filter and transfer to the aluminium-weighing dish. Dry, cool and weigh as in *b* above.

Calculation

$$mg\ Total\ Suspended\ \ Solids\ /\ L = \frac{(A - B) \times 1000}{mL\ sample}$$

where:

A = weight of filter + dried residue, mg, and
B = weight of filter, mg

TS	SOLIDS, TOTAL
Method:	**GRAVIMETRIC**
ID: ***1.25***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Evaporating dishes*, 100 mL capacity of porcelain, platinum or high-silica glass make

b. *Drying oven*, 104 ± 1°C

c. *Desiccator*

d. *Magnetic stirrer*

Reagents

Procedure

a. *Dry evaporating dish* at 104 ±1°C for 1 h, cool and store in a desiccator. Weigh immediately before use.

b. *Stir sample* with a magnetic stirrer. While stirring, pipette a measured volume into the pre-weighed evaporating dish using a wide bore pipette. Choose a sample volume to yield between 10 and 200mg dried residue. Evaporate to dryness in an oven at 104 ±1°C. If necessary add successive portions to the same dish after evaporation. To prevent splattering, the oven temperature may be lowered initially by 2°C below boiling point and raised to 104°C after evaporation for 1 h. Cool in a desiccator and weigh.

Calculation

$$\text{mg Total Solids /L} = \frac{(A - B) \times 1000}{\text{mL sample}}$$

where:
A = weight of dish + residue, mg
B = weight of dish, mg

TDS	SOLIDS, TOTAL DISSOLVED
Method:	**CALCULATION FROM TS AND TSS**
ID: ***1.40***	***Version:*** *1*

Standard Analytical Procedure

Procedure

Measure Total Solids, TS (method 1.25) and Total Suspended Solids, TSS (method 1.24) and obtain the data.

Calculation

$$TDS = TS - TSS$$

where:

TDS	=	Solids, Total Dissolved, mg/L
TS	=	Solids, Total, mg/L
TSS	=	Solids, Total Suspended, mg/L

Note

A laboratory should **not** report TDS values if these values are calculated from EC values! The multiplication factor may not be valid for each sample since it may change over time.

TDS	SOLIDS, TOTAL DISSOLVED
Method:	**GRAVIMETRIC AFTER FILTRATION**
ID: ***1.23***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. *Evaporating dishes*, 100 mL capacity of porcelain, platinum or high-silica glass made

b. *Drying oven*, 104 ± 1°C

c. *Desiccator*

d. *Magnetic stirrer*

e. Glass-fibre filter disk, Whatman grade 934 AH, Gelman type A/E, Millipore type AP4O or equivalent, diameter 2.2 to 12.5 cm.

f. Filtration apparatus, Membrane filter funnel or Gooch crucible with adapter and suction flask of sufficient capacity for sample size selected

Procedure

a. Wash filter paper by inserting it in the filtration assembly and filtering 3 successive 20 mL portions of distilled water. Continue suction to remove all traces of water. Discard washings.

b. Dry evaporating dish at 104 ± 1^0C for 1 h, cool and store in desiccator. Weigh immediately before use.

c. Stir sample with a magnetic stirrer and while stirring pipette a measured volume on to the filter using a wide bore pipette. Choose sample volume to yield between 10 and 200 mg dried residue. Wash with three successive 10 mL volumes of distilled water. Continue suction for about 3 min after filtration is complete.

d. Transfer total filtrate with washings to a weighed evaporating dish and evaporate to dryness in an oven at 104 ± 1^0C. If necessary add successive portions to the same dish after evaporation in order to yield between 10 and 200 mg dried residue. To prevent splattering oven temperature may be lowered initially by 2^0C below boiling point and raised to 104 ^{0}C after evaporation for 1h. Cool in a desiccator and weigh.

Calculate

$$\text{mg Dissolved Solids/L} = \frac{(A - B) \times 1000}{\text{mL sample}}$$

where:

A = weight of dried residue + dish, mg
B = weight of dish, mg.

SO4	SULPHATE
Method:	**NEPHELOMETRY**
ID: ***1.26***	***Version:*** *2*

Apparatus

a. *Nephelometric turbidity meter* with sample cells. Alternatively a spectrophotometer for use at 420nm with a light path of 2.5 to 10 cm

b. *Magnetic stirrer*

c. *Timer* with indication of seconds

Reagents

a. *Buffer solution A*: Dissolve 30g magnesium chloride, $MgCl_2.6H_2O$, 5g sodium acetate, $CH_3COONa.3H_2O$, 1g potassium nitrate, KNO_3, and 20 mL acetic acid CH_3COOH (99%) in 500 mL distilled water and make up to 1000 mL.

b. *Buffer solution B*: Only required if sample SO_4^{2-} concentration is less than 10 mg/L. Prepare as buffer solution A and add 0.111g sodium sulphate, Na_2SO_4.

c. *Barium chloride,* $BaCl_2$, crystals, 20 to 30 mesh

d. *Standard sulphate solution:* Dilute 10.4 mL standard 0.02*N* H_2SO_4 in to 100 mL (1.00 mL = 100μg SO_4^{2-}).

e. *Standard sodium carbonate, approximately 0.05N. Dry 3 to 5g sodium carbonate, Na_2CO_3, at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2g to the nearest mg, dissolve in distilled water and make to 1L.*

f. *Standard H_2SO_4, approximately 0.1N*. Dilute 2.8 mL conc. s*ulphuric acid to* 1L. Standardise against 40.00 mL 0.05*N* Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 5. Lift out electrodes, rinse into the same beaker and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker and finish titration to pH 4.3. Calculate normality of sulphuric acid:

$$\text{Normality, N} = \frac{A \times B}{53.00 \times C}$$

where:
A = g Na_2CO_3 weighed into the 1-L flask for Na_2CO_3 standard (see e.)
B = mL Na_2CO_3 solution taken for standardisation titration
C = mL acid used in standardisation titration

g. In case potentiometric titration is not possible use bromcresol green indicator to complete the titration. The indicator is prepared by dissolving 100mg bromcresol green sodium salt in 100 mL distilled water.

h. *Standard sulphuric acid, 0.02N*. Dilute the approximate 0.1*N* solution to 1L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

where:

N = exact normality of the approximate 0.1N solution.

Procedure

a. Standardise nephelometer following manufacturer's instructions.
b. Measure the turbidity of *sample-blank*, a sample in which no $BaCl_2$ is added.
c. Measure 100 mL sample, or a suitable portion made up to 100 mL, into a 250 mL conical flask. Add 20 mL buffer solution and mix. While stirring add a spoonful of $BaCl_2$ crystals. Stir for 60 $\pm$ 2 s.
d. Measure turbidity of the sample at 5$\pm$0.5 min after stirring ended
e. Prepare SO_4^{2-} standards at 5 mg/L increments in the range of 0- to 40 mg/L SO_4^{2-} according to the following protocol:

SO_4^{2-}, mg/L	5	10	20	30	40
Standard SO_4^{2-} solution, mL	5	10	20	30	40
Distilled water, mL	95	90	80	70	60

f. Develop $BaSO_4$ turbidity for the standards as above.
g. Determine turbidity of the standards using procedure as above and draw calibration curve between turbidity and SO_4^{2-} concentration, mg/L.
h. In case of buffer solution B is used for samples containing less than 10mg/L SO_4^{2-}, run a *reagent-blank* with distilled water in place of sample, developing turbidity and reading it as above.

Calculation

In case buffer solution A is used, read SO_4^{2-} concentration for the sample from the calibration curve after subtracting the turbidity of sample-blank from the turbidity of the treated sample. If less than 100 mL sample was used, multiply the result by 100/mLsample volume.

In case buffer solution B is used, for samples containing less than 10mg/L sulphate, calculate SO_4^{2-} as follows. Read SO_4^{2-} concentration in the treated sample from the calibration curve after subtracting the turbidity of sample-blank from the turbidity of the treated sample (same as in *i*). Subsequently read SO_4^{2-} concentration for the reagents from the turbidity value of the reagent-blank (see procedure '*h'*) from the calibration curve. Report the corrected SO_4^{2-} concentration in the sample after subtracting the reagent-blank SO_4^{2-} concentration from the sample SO_4^{2-} concentration.

T	TEMPERATURE
Method:	**MERCURY THERMOMETER**
ID: ***1.27***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Mercury thermometer having a scale marked for every 0.1^{o}C.

Procedure

a. Immerse thermometer in the sample up-to the mark specified by the manufacturer and read temperature after equilibration.

b. When a temperature profile at a number of different depths is required a thermistor with a sufficiently long lead may be used.

TURB	TURBIDITY
Method:	**NEPHELOMETRIC**
ID: ***1.28***	***Version:*** *1*

Standard Analytical Procedure

Apparatus

a. Nephelometric turbidity meter with sample cells

Reagents

a. *Solution I.* Dissolve 1.000g hydrazine sulphate, $(NH_2)_2.H_2SO_4$ in distilled water and dilute to 100 mL in a volumetric flask.

b. *Solution II.* Dissolve 10.00g hexamethylenetetramine, $(CH_2)_6N_4$, in distilled water and dilute to 100 mL in a volumetric flask.

c. *4000 NTU suspension*. In a flask mix 5.0 mL of Solution I and 5.0 mL of Solution II. Let stand for 24 h at 25 ±3°C. This results in a 4000 NTU suspension. Store in an amber glass bottle. The suspension is stable for up to 1 year.

d. Dilute 4000 NTU stock solution with distilled water to prepare dilute standards just before use and discard after use.

Procedure

a. *Calibrate nephelometer* according to manufacturer's operating instructions. Run at least one standard in each instrument range to be used.

b. *Gently agitate sample*. Wait until air bubbles disappear and pour sample into cell. Read turbidity directly from instrument display.