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DELFT HYDRAULICS with
HALCROW, TAHAL, CES,
ORG & JPS

VOLUME 7
WATER QUALITY ANALYSIS

OPERATION MANUAL

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1 INTRODUCTION

The manual on Hydrological Information Systems consists of several volumes. Volume 7 deals with “Water Quality Analysis”: and consists of two parts:

1. **Design Manual**, in which basic principal procedures are put in context.
2. **Operation Manual**, dealing with operational procedures.

Volume 7 of the Operation Manual describes procedures to be followed for ‘Water Quality Analysis’ in the laboratories. This manual pertains to both surface water and groundwater quality analysis. Although groundwater quality analysis does not include certain parameters such as chlorophyll-a and other microbiological parameters, some groundwater laboratories also conduct analysis of surface water samples. Therefore this manual includes all information which is relevant for analysis of water quality. It is set up as follows:

- Chapter 2, ‘Analysis Protocol’ discusses the quality assurance aspects of analysis, which each laboratory must follow. It includes good laboratory practices and procedures for maintaining within-laboratory analytical quality control.
- Chapter 3 gives the formats for sample receipt, storage and work distribution.
- The recommended standard analytical procedures are given in Chapter 4.
- Chapters 5 and 6 describe procedures for reporting of the analysis results and data recording and validation, respectively.

2 ANALYSIS PROTOCOL

The importance of the quality assurance (QA) is recognised in any water quality monitoring programme. The QA programme is an essential part of analytical work. Each analyst as well as a laboratory organisation should follow the established procedures to detect and to correct problems and take every reasonable step needed to keep the measurement process reliable.

The more important features of a QA programme should include:

- the use of documented methods of analyses - all methods are documented in the Hydrology Project report '*Guidelines on Standard Analytical Procedures for Water Analysis*'. These are also given in Chapter 4.
- properly maintained and calibrated equipment
- properly trained staff
- effective internal quality control (Within-laboratory analytical quality control (AQC), using Shewhart control charts)
- participation in periodic programmes for evaluation of measurement bias (Inter-laboratory AQC)
- external assessment by accreditation or other compliance schemes

The basic elements of quality assurance must be followed and enforced to obtain reliable and comparable results.

2.1 GOOD LABORATORY PRACTICE

A number of laboratory operations and precautions related to analysis of water quality parameters must be routinely performed in a laboratory to obtain reliable information. These practices are termed *good laboratory practices*. Some analyses, that measure extremely small level of contaminants using use advanced level instrumentation, would require special precautions. Practices that are to be followed routinely and are basic in nature to all determinations are described here.

2.1.1 CHEMICALS AND REAGENTS

Purity of reagents has an important bearing upon the accuracy that can be attained in an analysis. Commercially available chemicals are routinely classified as:

- technical grade
- laboratory or analytical reagent grade
- primary-standard grade
- special purpose reagents

Technical grade reagents, in general, are not used in a laboratory. Where bulk quantities may be required or when purity is not of major concern, such as preparation of chromic acid cleaning solution, technical grade reagents may be used.

Routine analyses in a water testing laboratory may be performed mostly using laboratory or analytical reagent grade chemicals. Where primary standards are to be made, primary standard grade reagent should be used.

Special purpose reagents are required for analyses when micro-level contaminants are measured through atomic absorption spectroscopy and gas chromatography.

The following rules should always be followed while handling reagents and chemicals:

- As far as possible, use the smallest packing of chemical that would supply the desired quantity.
- Replace the cap or stopper of the bottle immediately after taking out your requirement.
- Stoppers of reagent bottles should never be placed on the desktop.
- Do not insert spatulas or pipettes in reagent bottles. Take out a slightly excess amount in another container from where uses the required quantity.
- Never return any excess chemical or reagent back to the bottle.
- Some reagents require special storage conditions, such as dark colour bottles for light sensitive chemicals or low temperature solvents and reagents subject to microbial degradation.
- Do not use a reagent after the recommended shelf life.
- Dry solid chemicals for making solutions, in a suitable container, as directed in the standard analytical procedure for the determination.

2.1.2 CLEANING OF GLASSWARE

Volume calibrations are based on clean volumetric equipment. Cleanliness of volumetric glassware is, therefore, particularly important if calibration is to have any meaning. Only clean glass surfaces will support a uniform film of liquid; the presence of dirt or oil will tend to cause breaks in this film. The existence of breaks is a certain indication of an unclean surface. A brief soaking in warm detergent is usually sufficient to remove the grease and dirt responsible for causing the water breaks.

Where detergent is not effective, rinse glassware, except that used for chromium and manganese analysis, with a cleaning mixture made by adding 1 L of conc. H_2SO_4 slowly with stirring, to 35 mL saturated sodium dichromate solution. Rinse with other concentrated acids to remove inorganic matter.

Use detergents or conc. HCl for cleaning hard rubber and plastic bottles.

After the glassware and bottles have been cleaned, rinse thoroughly with tap water and finally with distilled water. Glassware can be dried by placing inverted on a drying rack. After drying, glassware should be stored in a clean and protected dust-proof cabinet.

2.1.3 DISTILLED OR REAGENT WATER

Distilled water may be classified on the basis of its electrical conductivity (EC) as follows:

Type	EC, $\mu mho/cm$
I	< 0.1
II	< 1
III	< 10

Laboratories are provided with stainless steel water distillation stills. Distilled water obtained from such stills is of adequate purity (Type III) for making reagent solutions for routine analyses carried out in water testing laboratories and cleaning of glassware. However, in case of analyses for micro-level contaminants better quality distilled water (Type I or II) may be needed.

Ion exchange columns and double distillation, all-glass water stills, are used to obtain Type I and II distilled water, respectively. Type I distilled water is also called de-ionised water.

2.1.4 MEASUREMENT OF MASS OR WEIGHING

Accurately measuring mass of substances is a fundamental requirement for almost all types of analyses. In the laboratory, the mass is determined by comparing the weight of an object with the weight of a set of standard masses using a balance. Because acceleration due to gravity affects both the known and unknown to the same extent, equality of weight indicates equality of mass. The terminological distinction between mass and weight is, therefore, seldom made.

Analytical balances, with an accuracy of ± 0.1 mg, are commonly used to determine the weight. For analyses for micro-level contaminants balances with a resolution of ± 0.01 mg may be used. It is of paramount importance that balances are used and maintained with great care. To avoid damage or minimise wear on the balance and to obtain accurate weights, adhere to the following rules:

- Be certain that the arresting mechanism of the beam is engaged whenever the loading on the balance is being changed and when the balance is not in use.
- Centre the load on the pan insofar as possible.
- Protect the balance from corrosion. Only non-reactive glass, plastic or metal objects should be placed directly on the pan. In case of volatile or corrosive substances, take special precautions of sealing it in a weighed glass ampoule.
- Do not attempt to adjust or repair the balance if you are not trained to do so.
- Keep the balance and its case scrupulously clean. A camel's hair brush is useful for cleaning any spilled material or dust.
- Keep silica gel inside the balance to absorb moisture, if any.
- Do not weigh an object that has been heated until it has returned to the room temperature.
- Do not touch a dried object with bare hands: use tongs to prevent the uptake of moisture.
- Always place the balance on a vibration free platform.

The drying of a chemical or a container to constant weight is the process in which the object is first heated at an appropriate temperature, ordinarily for an hour or more, following which it is cooled to room temperature and weighed.

Solids are conveniently dried and stored in weighing bottles of glass or plastic. Oven drying, usually at 105 to 110 °C is the more common way of removing the absorbed moisture. The dried objects are stored in a desiccator while cooling. The base section of the desiccator contains a quantity of a chemical drying agent, such as anhydrous calcium chloride, calcium sulphate, or anhydrous magnesium perchlorate.

2.1.5 RECORDING OF DATA

Each analyst should have a laboratory notebook to record measurements and observations concerning analyses. The notebook itself should be permanently bound with a hard cover. The pages should be consecutively numbered. The first few pages should be reserved for a table of contents which should be kept up to date.

- All data should be directly entered into the notebook.
- Entries should be labelled. Entries should not be crowded.
- Each notebook page should be dated as it is used.
- An erroneous entry should never be erased, obliterated or written over. Instead it should be crossed out with a single horizontal line and the correct entry should be located adjacent to it.
- Pages should never be removed from the notebook. It is sufficient to draw a single line diagonally across a page that is to be disregarded.

2.1.6 MAINTENANCE OF EQUIPMENT

A chemical laboratory is provided with a variety of equipment ranging from simple heating devices to extremely sophisticated computer-controlled analytical equipment. It is a good practice to keep a separate logbook for each major equipment where details of their use, maintenance schedule, breakdowns and repairs, accessories, supply of consumables, etc., are carefully entered. Such a record will help in properly maintaining the instrument and planning for future. For good functioning of the laboratory, it is important that all laboratory equipment, general items and analytical equipment are in good functioning order.

There are several reasons why preventive maintenance is needed:

- All equipment can get dirty & dusty with time
- Some components can wear out and need replacement
- Good maintenance assures good analytical results
- Regular maintenance is part of laboratory Quality Control
- Prevention is better than cure

Overview of Laboratory Equipment

Items available in the water quality laboratories can be categorised as general equipment, analytical equipment and meters:

General Laboratory Equipment	Analytical Laboratory Equipment	Meters
• air conditioner	• analyser kit (portable field measurements)	• conductivity meter
• steriliser, autoclave	• balances	• pH meter
• centrifuge	• distillation apparatus	• ion meter & specific ion probes
• desiccator	• COD digester	• DO meter
• deep freezer	• distillation apparatus, CN and F	• silto meter
• filtration assembly for membrane filters	• digestion and distillation unit, Kjeldahl	• flame photometer
• fume cupboard	• soxhlet extraction unit	• spectrophotometer
• hot plate – with magnetic stirrer	• water purifiers: distillation units and ion exchange resin column	• turbidity meter (nephelometer)
• ice box	• pipettes and dispensers	• gas chromatograph
• lpg connection	• glassware	• atomic absorption spectrophotometer
• ovens: general purpose and muffle furnace	• thermometers	
• refrigerator		
• shaker		
• stabiliser		
• tissue grinders		
• vacuum pump		
• water baths, general purpose and bacteriological		
• water heater (geyser)		
• fire extinguisher		

Table 2.1: Overview of Laboratory Equipment

General guidelines for laboratory operation and maintenance

There are a number of general guidelines which laboratory staff can follow for laboratory maintenance:

- **Keep the laboratory clean**
This is the single most important thing you can do to keep laboratory equipment in good working order. Dirt and dust in the air, on the lab bench or on the floor will work their way into the equipment and affect sensitive parts. Clean the lab benches and sweep the floor daily. Do not bring food or drinks on the lab benches.
- **Place dust covers on all analytical equipment when not in use**
Despite best efforts to keep the laboratory as clean as possible, there will be some dust and dirt present. Protect sensitive analytical equipment with dust covers at all times when they are not in use. Dust covers should not be kept on dusty floor/platform while the instrument is in use.
- **Designate a fixed location for each piece of analytical equipment**
Most items of laboratory analytical equipment do not respond well to frequent transfers. Each piece of equipment should have a designated place in a clean, dry and stable location. You should avoid moving equipment or packing and unpacking items from storage.
- **Have a maintenance contract for each of analytical equipment**
Even the best-cared-for equipment need periodic maintenance, either by lab staff or a specialised external service. The best way to keep laboratory analytical equipment functioning well for many years is to have regular servicing by an external qualified technician. An Annual Maintenance Contract (AMC) with the equipment supplier will cover regular maintenance checks by a technician as well as servicing when a specific problem occurs.
- **Keep a maintenance logbook for each item of analytical equipment**
 1. list schedule for maintenance activities to be followed in the laboratory
 2. list schedule for replacing equipment parts
 3. register all regular maintenance checks
 4. register all equipment problems and repairs

In this way the working history of each item will be available. The logbook should be readily available near the equipment item. The responsible person should sign the maintenance logbook when any maintenance activity is undertaken.

- **Have equipment manual and troubleshooting list available for each item of analytical equipment**
If there is trouble with an item of equipment, a designated person should try to identify the problem. Upkeep of each laboratory item should be the responsibility of one person. There should be a copy of the manual and a troubleshooting list kept together with the maintenance logbook, near the equipment item.
- **Have a set of tools and maintenance equipment available in the laboratory**
Basic tools and equipment cleaning and maintenance supplies should be available in the laboratory. Items suggested include: small brushes, screwdrivers, extra fuses, voltmeter, tweezers, etc.
- **Don't try anything difficult**
If there is a serious problem with an item of equipment, the service contractor should be contacted. Opening a sensitive piece of equipment by unqualified person can make the problem worse. Make simple checks as outlined in the troubleshooting list, e.g. check power supply or equipment fuse. Do not open or dis-assemble equipment yourself.

- **Maintenance of equipment**

In addition to the general maintenance suggestions made above, some suggestions for specific items are listed below, as to what can be done in the laboratory:

Air conditioner: Keep the air filter clean and dust free.

Glassware: All glassware should be cleaned on a regular basis as described in Section 2.1.2.

Distilled Water Apparatus: The distilled water apparatus for making high purity water needs regular control and maintenance. In the operation of water stills care should be taken to run the condenser cooling water whenever the still is in use. All stills are prone to scaling and accumulation of precipitated salts which, if not attended to, results in carry over of salts in distillate and will ultimately lead to overheating to cause damage to the heating elements. The scales should be periodically removed by dissolving in dilute solution of HCl.

In the case of ion exchange columns, a record should be kept of the volume of the product water and its EC value. The resin should be regenerated as per the supplier's instruction when the EC value exceeds the prescribed limit.

Desiccator: A desiccator is a special covered glass contained designed for storage of objects in a dry atmosphere. It contains a desiccant, which acts as a drying agent and can be heated to restore its drying powers. Air tight seal is obtained by a flat glass edge with acid free grease. The edge must be cleaned and greased periodically.

Deep freezer, icebox, refrigerator: Periodically empty, defrost if applicable and thoroughly clean.

Balances: A number of operation and maintenance procedures were listed in Section 2.1.4. These should be strictly followed to preserve the optimal operation of the balance.

Conductivity meter: Electrodes have a limited life-span and will need replacement when the cell constant starts changing significantly or the readings become erratic.

pH meter: The pH electrode needs to be rinsed thoroughly after each day of use to remove residuals that may accumulate on the probe, e.g. alkali build-up. The glass membrane electrode needs periodic filling with KCl reference solution. Proper storage is important. The pH meter can be permanently set up on the bench, with the electrode immersed in distilled water.

Ion meter, probes & specific ion electrodes: The specific ion electrodes should be rinsed after each day of use to remove residuals that may accumulate. The electrodes may need periodic filling with reference solution. Proper storage is important. If the ion meter is used regularly, it can be permanently set-up on the bench, with the electrodes immersed in distilled water. It should be recognised that the electrodes have a limited life-span and will need to be replaced every few years, as specified by the manufacturer. Cleaning and careful handling will prolong the life-span.

Many of the major equipment, such as, **flame photometer, spectrophotometer, gas chromatograph, atomic absorption spectrophotometer**, need to be maintained in accordance with the manufacturer's instructions. It is preferable to enter into AMC for some of these, which should include an annual check.

2.1.7 SAMPLE COLLECTION AND PRESERVATION

All laboratories interact with field staff who carries out sampling, conduct site analyses and transports samples. The staff of the chemical laboratory must advise the field staff regarding procedures for on-site analyses and method of sample collection. The laboratory staff should also specify preservation technique of samples for different analyses to be carried out in the laboratory and supply reagents required for preservation. The laboratory staff should make it a practice to periodically visit the sampling sites, observe the procedures being carried out and advise as necessary.

Prior to each sampling campaign, the laboratory staff will prepare the correct number and type of bottles necessary, and review the sampling itinerary with the field staff.

Sampling procedures, different types of samplers, preservation, procedures, etc., are described in Volume 6 of the Design and Field Manuals.

2.1.8 LABORATORY SAFETY

All laboratory employees must make every effort to adhere to certain basic safety rules to protect themselves and their fellow workers. The sources of hazard in a laboratory are corrosive and poisonous chemicals, broken glass, explosion, fire and electrical shock. Some common safety rules are given below. In case a health and safety programme has been developed for your laboratory, you should always follow it.

- Learn the locations of eye fountain, emergency shower, fire blanket and fire extinguisher.
- Eye protection must be worn at all times.
- In handling all chemicals, avoid contact with skin. In the event of such contact, immediately wash the affected area with copious amounts of water.
- Avoid working alone in a laboratory if the procedures to be conducted are hazardous.
- Do not drink, eat or smoke in areas where laboratory chemicals are present. Do not drink from laboratory glassware.
- Do not store food or beverages in storage areas and refrigerators that are used for laboratory operations.
- Always use a suction bulb to draw chemicals in a pipette. Never use the mouth to provide suction.
- Be extremely tentative in touching the objects that have been heated.
- Always fire polish the ends of freshly cut glass tubing. Never attempt to force glass tubing through a hole in the stopper. Instead, make sure that both the tubing and the hole are thoroughly wet with soapy water and protect hands with towel or heavy gloves.
- Use fume hoods where toxic or noxious gases or fumes are likely to be evolved.
- Use care in testing for odours; use the hand to waft vapours above containers towards nose.
- In some locations it may not be permissible to flush heavy metals or poisonous substances down the drain. In case of such restrictions, alternative arrangements are required.

2.1.9 ANALYTICAL QUALITY CONTROL

To ensure the quality of the sampling and chemical analysis, control tests can be carried out. Specific control tests to be followed are:

- Run control standards along with the regular sample load and record their data on a Shewhart Chart.
- Routine tests on the effectiveness of the cleaning of sampling equipment and sample containers. Field blanks, samples of de-ionised or distilled water that are taken into the field and treated as samples, to check on contamination.
- Field check samples to provide routine checks on sample stability. Checks can be done by dividing a real sample into two and making a known addition to one portion. The extent of recovery is a check whether the preservation, sample transport and storage are satisfactory or not.

This section describes only running of control standards using Shewhart charts.

Running control standards does not ask a laboratory to perform replicate analysis on samples taken from the field. Quality control test for precision is performed on control samples prepared by the laboratory that carries out the exercise.

Preparation of control samples

As an example, the composition and the preparation of six control solutions for eight parameters is described in Table 2.2. A laboratory may define its own solutions for other water quality parameters.

Use reagents of high purity (primary-standard grade). Except as noted, dry all salts at 105°C for 1 h and store in desiccator before weighing. Use de-ionised (Type I) water, if available.

Boron	Since boric acid loses weight on drying, do not dry at 105 °C. Keep the reagent bottle tightly stoppered. Dissolve 571.6 mg anhydrous boric acid, H ₃ BO ₃ , in distilled water and dilute to 1000 mL. Dilute 10 mL to 1000 mL to give a working control solution of 1 mg/L B.
Electrical conductivity	Dissolve 1.491g potassium chloride, KCl, in distilled water and dilute to 1000 mL. Dilute 100 mL to 1000 mL to give a working control solution of 296 µmho/cm conductivity at 25 °C.
Fluoride	Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL. Dilute 20 mL to 1000 mL to give a working control solution of 2 mg/L F ⁻ .
Phosphate	Dissolve 219.5 mg anhydrous potassium di-hydrogen phosphate, KH ₂ PO ₄ , in distilled water and dilute to 1000mL. Dilute 20 mL to 100 mL to give a working control solution of 10 mg/L PO ₄ ³⁻ - P.
Sodium	Dissolve 0.1479 g anhydrous sodium sulphate, Na ₂ SO ₄ , in distilled water and dilute to 1000 mL. Dilute 20 mL to 100 mL to give a working control solution 9.58 mg/L Na ⁺ .
Sulphate	Use the working control solution for Sodium that has a working control solution of 20 mg/L SO ₄ ²⁻ .
Total hardness	Take 1.000 g anhydrous calcium carbonate CaCO ₃ in 500 ml. conical flask. Place a funnel on the neck of the flask and add drop by drop slowly 1 + 1 HCl until all the CaCO ₃ has dissolved. Add 200 ml distilled water and boil for a few minutes to expel CO ₂ . Cool and add few drops of methyl red indicator and adjust to the intermediate orange colour by adding 3N NH ₄ OH or 1 + 1 HCl as required. Transfer quantitatively to a 1 L volumetric flask and make up to the mark with distilled water. Dilute 10 ml to 100 ml to give working control solution of 100 mg/L total hardness as CaCO ₃ .
Total solids	Use the working control solution for Electrical Conductivity that has total solids concentration of 149 mg/L.

Table 2.2: Preparation of control samples

Construction of Shewhart charts

The Shewhart control chart is the most widely used form of control charts. In its simplest form, results of individual measurements made on a control sample are plotted on a chart in a time series. The control sample is analysed in the same way as the routine samples at fixed time intervals. The following procedure is used for the construction of the charts.

- Analyse three replicates of a standard solution for the desired parameter on each day for eight days over a 12 day period. (The number of replicate analyses should thus be 24 carried out over a 12 day period). Record the values on the proforma given in Figure 2.1.
- Performing all replicate analysis in a shorter period will result in more precise results, however, this high precision may, over a longer period, not be realistic. Use standardised methods for the analysis.
- Calculate mean (\bar{x}) and standard deviation(s) based on the results of the analysis. Use copies of pro forma given in Figure 2.1. Subsequently calculate upper and lower control limits (UCL and LCL) and upper and lower warnings limits (UWL and LWL) from the mean and standard deviation at $\pm 2\sigma$ and $\pm 3\sigma$ from the mean, respectively.
- Construct the Shewhart chart by plotting horizontal lines representing the control lines in the blank Shewhart chart given in Figure 2.2. Keep the chart with the instrument used in the analysis of the parameter. The above steps are to be carried out simultaneously for each parameter.
- Individual points of these analyses should not be plotted.
- After plotting the control lines on the blank Shewhart chart, repeat the analysis of the standard solution 2 to 3 times per week or after 20 to 30 routine samples, whichever comes first. Plot the result of these analyses on the chart.

- Keep on using this chart until it is filled, that means there is no more space left on the horizontal axes to plot analytical results of the control sample. Calculate the UCL, UWL, Mean, LWL and LCL based on the latest 20 analysis plotted on the Shewhart Control Chart. Construct a new Shewhart chart by plotting horizontal lines representing the UCL, UWL, Mean, LWL and LCL, as explained above.
- Keep the chart with the instrument used in the analysis of the parameter until it is full again.

Interpretation of Shewhart Control Charts

If a set of analytical results is obtained for a control sample under conditions of routine analysis, some variation of the observed values will be evident. The information is said to be statistically uniform and the analytical procedure is said to be under statistical control if this variation arises solely from random variability. The function of a control chart is to identify any deviation from the state of statistical control. This type of chart provides a check on both random and systematic error gauged from the spread of results around the mean and any trend in their displacement, respectively. Examples of data plotted on Shewhart charts are shown in Figure 2.3.

Control Limits: UCL and LCL are set at: $\text{Mean} \pm 3\sigma$. Assuming the results for the control sample follow the Normal frequency distribution, it would be expected that only 0.3% of results would fall outside the lines drawn at 3 standard deviations above and below the mean value. Individual results would be expected to fall outside these limits so seldom (3 out of 1000 results), that such an event would justify the assumption that the analytical procedure was no longer in statistical control, i.e., a real change in precision has occurred.

Warning Limits: UWL and LWL are set at: $\text{Mean} \pm 2\sigma$. If the method is under control, approximately only 4.5% of results may be expected to fall outside these lines.

Standard Methods list the following actions that may be taken based on analysis results in comparison to the standard deviation.

Control limit: If one measurement exceeds the limits, repeat the analysis immediately. If the repeat is within the UCL and LCL, continue analyses; if it exceeds the action limits again, discontinue analyses and correct the problem.

Warning limit: If two out of three successive points exceeds the limits, analyse another sample. If the next point is within the UWL and LWL, continue analyses; if the next point exceeds the warning limits, discontinue analyses and correct the problem.

Standard deviation: If four out of five successive points exceed one standard deviation, or are in increasing or decreasing order, analyse another sample. If the next point is less than one standard deviation away from the mean, or changes the order, continue analyses; otherwise discontinue analyses and correct the problem.

Central line: If six successive points are on one side of the mean line, analyse another sample. If the next point changes the side continue the analyses; otherwise discontinue analyses and correct the problem.

Sources of error in specific analyses

Examples of specific sources of error for the analyses of TDS, TH, EC, fluoride, sulphate, phosphate, nitrate, sodium and boron are given below (Table 2.3). A laboratory should keep its own record for other variables.

Potential sources of error in TDS analysis	
Errors may be in the balance or oven:	<ul style="list-style-type: none"> balance precision and calibration unstable base for balance drying temperature and temperature control defect in oven
Errors may be in weighing procedures	<ul style="list-style-type: none"> poor handling of glassware/paper, causing addition of weight from other sources weighing of dish without drying for tare weight. weighing while dish is still hot (air turbulence) poor procedures in using balance, e.g. not closing door before taking reading
Other sources of error	<ul style="list-style-type: none"> small amounts of solid dried results in accumulating errors due to differential method large amount of solids (water-trapping crust formation) hygroscopic solids require prolonged drying poor storage during cooling in dessicator loss of sample during handling improper labelling of sample calculation error
Potential sources of error in TH analysis	
Error from preparation of reagents:	<ul style="list-style-type: none"> decontamination of the glassware by proper washing and rinsing drying of chemical powders proper weighing (balance precision) preparation of dilutions (volumetric flask) distilled water (purity)
Error in Titration	<ul style="list-style-type: none"> standardisation of titrant on day of analysis using primary standard reading of burette during titration: a sample volume between 5 and 20mL is optimal for a 50mL (0.1mL readability) reagent blank correction (titration of distilled water) indicator in blank and standard should be of same quality determination of endpoint: colour change (light conditions, white background) determination of endpoint: speed of adding the last drops correct pH (buffer solution) during titration If completion of titration takes more than 5 minute, calcium carbonate may precipitate and thus reduce the concentration calculation errors
Potential sources of error affecting in EC measurement	
Error may be in the instrument:	<ul style="list-style-type: none"> condition of the conductivity cell sensitivity of instrument age of instrument
Error may be in accuracy of preparation of KCl calibration solution (0.01M KCl):	<ul style="list-style-type: none"> drying of KCl powder weighing (defective balance) quality of the de-mineralised water used for the calibration solution glassware used (volumetric flask vs. measuring cylinder)
Error may be in measurement procedure:	<ul style="list-style-type: none"> calibration of the instrument (cell constant adjustment) measurement of the sample temperature and temperature correction decontamination of the probe
Error may be in other general procedures:	<ul style="list-style-type: none"> decontamination of glassware wrong labelling of sample calculation and reporting errors
Probable source of errors in Fluoride measurement	
Error in preparation of reagents	<ul style="list-style-type: none"> Decontamination of the glasswares by proper washing and rinsing Drying of chemicals powders Proper weighing (defective balance) Distilled water (purity) Glasswares used (vol. flask v/s measuring cylinder)
Error due to presence of interfering ions	<ul style="list-style-type: none"> Alkalinity Aluminium chloride chlorine colour and turbidity iron phosphate sulphate (distillation is necessary)

Other errors:	<ul style="list-style-type: none"> • Sample storage should be done in polyethylene bottle • Dechlorinate with sodium arsenite rather than sodium thiosulphate
Probable source of errors in Sulphate measurement	
Error in preparation of reagents	<ul style="list-style-type: none"> • Decontamination of the glasswares by proper washing and rinsing • Drying of chemicals powders • Proper weighing (defective balance) • Distilled water (purity) • Glasswares used (vol. flask v/s measuring cylinder)
Error in Turbidimetry measurement	<ul style="list-style-type: none"> • Improper measurement • Sensitivity of the instrument • Uneven light path in cuvette • Uneven distribution of the solids (magnetic stirrer is required to homogenise the sample)
Other sources of error:	<ul style="list-style-type: none"> • Presence of organic matter may induce growth of sulphate reducing bacteria • Presence of suspended matter, colour and silica (leading to high values), and of alkali metal sulphate (leading to low values) • Wrong identification of sample • Wrong calculation
Probable source of errors in Nitrate - N measurement	
Preparation of reagents	<ul style="list-style-type: none"> • Decontamination of the glasswares by proper washing and rinsing • Drying of chemicals powders • Proper weighing (defective balance) • Distilled water (purity) • Glasswares used (vol. flask v/s measuring cylinder)
Error due to presence of interfering chemicals (add EDTA to eliminate)	<ul style="list-style-type: none"> • Chloride • Bicarbonate • Cyanide • Sulphide • Halides
Other:	<ul style="list-style-type: none"> • Take all precautions applicable to colorimetry • Remove residual chlorine if any
Probable source of errors in Phosphate – P measurement	
Error in preparation of reagents	<ul style="list-style-type: none"> • Decontamination of the glasswares by proper washing and rinsing • Drying of chemicals powders • Proper weighing (defective balance) • Distilled water (purity) • Glasswares used (vol. flask v/s measuring cylinder)
Other sources of error:	<ul style="list-style-type: none"> • Selection of method of analysis • Sample preparation including filtration, digestion etc • Improper preservation of sample (do not preserve in acid or chloroform) • Storage in plastic bottle
Probable source of errors in Sodium measurement	
Error in preparation of reagents	<ul style="list-style-type: none"> • Decontamination of the glasswares by proper washing and rinsing • Drying of chemicals powders • Proper weighing (defective balance) • Distilled water (purity) • Glasswares used (vol. flask v/s measuring cylinder)
Error with Flame photometer	<ul style="list-style-type: none"> • Interference due to presence of potassium and calcium • Chloride, sulphate and bicarbonate may cause radiation interference • Clogging particles
Probable source of errors in Boron measurement	
Error in preparation of reagents	<ul style="list-style-type: none"> • Decontamination of the glasswares by proper washing and rinsing • Drying of chemicals powders • Proper weighing (defective balance) • Distilled water (purity) • Glasswares used (vol. flask v/s measuring cylinder)
Other sources of error:	<ul style="list-style-type: none"> • Nitrate concentration above 20 mg/l interfere the measurement. • High results are possible when hardness is exceeding 100 mg/l • Impurity in curcumin is a common source of error • Improper spectrophotometric measurements

Table 2.3: Potential sources of error for some water quality analyses

Errors that cannot be detected by within-laboratory AQC

The within-laboratory AQC exercise focuses mainly on precision. A laboratory on its own cannot detect many sources of bias. A good example to illustrate this is the total hardness method. If the analytical balance in a lab always reads 10% in excess, all solutions prepared will have a 10% higher concentration: the Standard CaCO₃ solution, the EDTA titrant and also the control sample containing CaCO₃. This error can only be detected by analysing a sample prepared by a laboratory with a correctly functioning balance. The current laboratory will underestimate the concentration of such a inter-laboratory sample by 10% because their EDTA titrant is '10% too strong'.

As a minimum goal for precision, however, the precision that can be obtained by correctly and adequately following the method prescribed by the APHA Standard Methods for the examination of water and wastewater may be adopted

In some cases freshly introduced bias may be detected. For example, if the measurements consistently fall on one side of the previously calculated mean, it indicates a freshly introduced bias.

An inter-laboratory AQC exercise should be conducted for detecting bias or accuracy for analysis, see Design Manual, Volume 7 on water quality analysis, Chapter 8.

Parameter Replicates	TS	TH	EC				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
Mean							
Standard Deviation							
UWL (x+2sd)							
LWL (x-2sd)							
UCL (x+3sd)							
LCL (x-3sd)							

Figure 2.1: Blank form for the set-up of a Shewart Chart for new variable (initial control lines)

Laboratory / Organisation:																													
Address:																													
Parameter																													
Control concentration:																													
Method:																													
Mean:																													
Standard deviation:																													
UWL:																													
UCL:																													
Measured Conc.																													
Date																													

Figure 2.2: Blank form to plot control lines (SHEWART CHART)

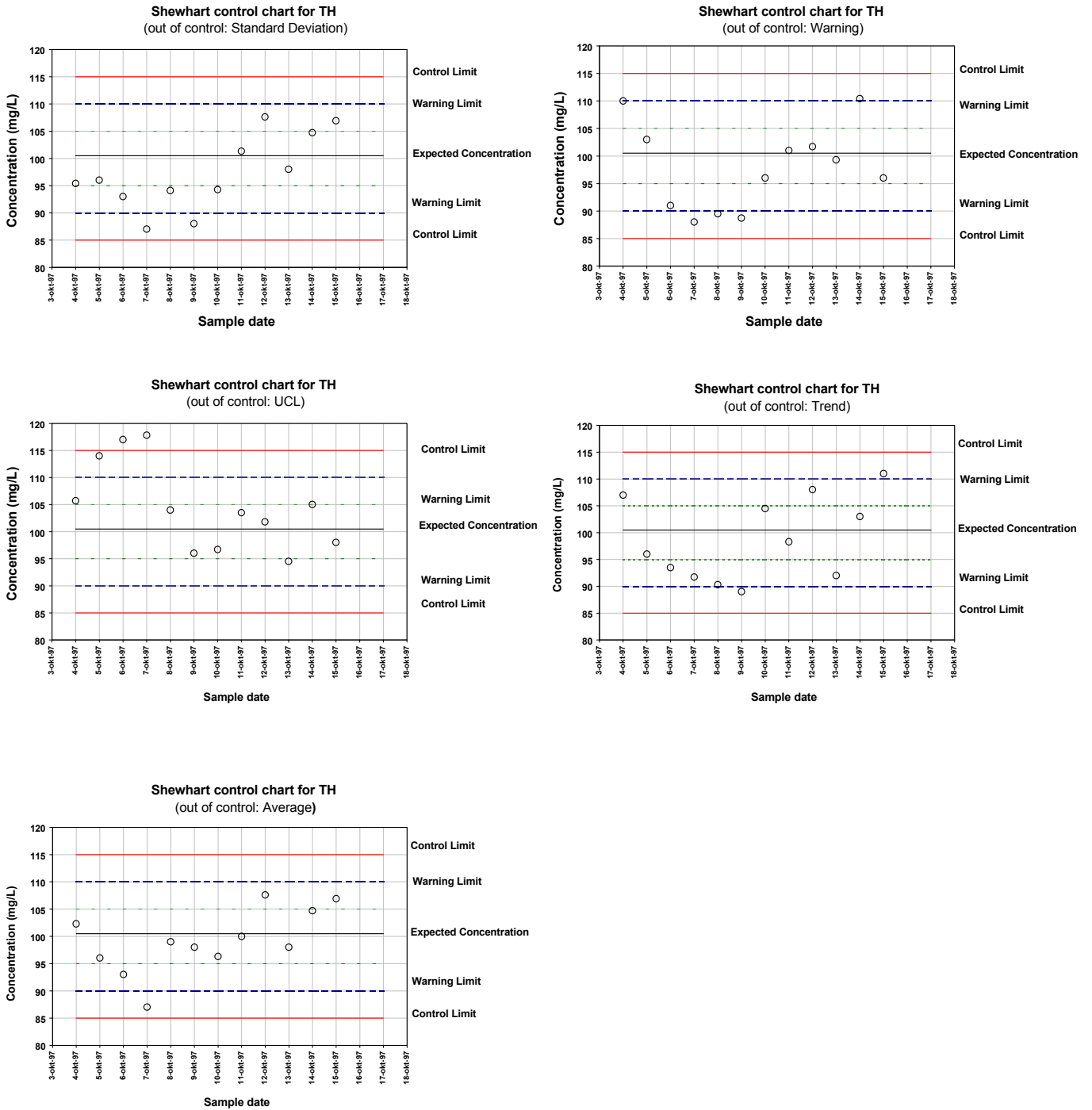


Figure 2.3: Example of loss of statistical control by different criteria

3 SAMPLE ANALYSIS

The field staff, who collects the water samples, hands over the samples to the laboratory staff for further analysis. It now becomes the responsibility of the laboratory to carefully identify the sample, store it properly and analyse it for the parameters according to the objectives of the sampling programme under which the sample was collected. Each sample should have a sample identification form (see Field Manual, Volume 6, Chapter 3), filled in by the field staff at the time of the collection of the sample, which should be surrendered to the laboratory where it should be kept in a master file. The information recorded on this will assist the laboratory in further processing the sample.

3.1 SAMPLE RECEIPT RECORD

Each laboratory should have a bound register, which is used for registering samples as they are received. An example of headings and information for such a register is given in Table 3.1.

- Column (3) gives the station code conventionally followed by the monitoring agency.
- Column (4) gives the project under which the sample is collected.
- Column (7) corresponds to the parameter(s) code given in the sample identification form.
- Column (8) gives the laboratory sample number assigned to the sample as it is received in the laboratory. Note that the numbering has two parts separated by a hyphen. The first part is assigned in a sequential manner as samples are received from various stations. If two samples are collected at the same time from a station for different sets of analysis, the first part of the number is the same. The second part corresponds to the parameter code.
- The results of the analyses of all the samples having the same first part of the code would be entered in the data entry system as one sample having the same station code and time of sample collection.

Date/Time received at laboratory	Date/Time collected	Station code	Project	Collecting agency/collector	Preservation	Parameter code	Lab. Sample No.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
02.07.99/1400	01.07.99/1100	M 22	WQ monitoring	SW Div II/ Singh	No	1	28-1
02.07.99/1400	01.07.99/1700	M 24	WQ monitoring	SW Div II/ Singh	No	1	29-1
02.07.99/1400	01.07.99/1700	M 24	WQ monitoring	SW Div II/ Singh	Yes	4	29-4
05.07.99/1100	02.07.99/1300	S 44	Survey A	SPCB/ Bhat	Yes	5	30-5

Table 3.1: Example of a sample receipt register

3.2 STORAGE

As a general rule, samples for water quality analysis should be stored at a temperature below 4° C and in the dark as soon after sampling as possible. This is to prevent unwanted changes in the determinand values. For this reason it is good practice to store all water samples in the refrigerator until they can be analysed. It is realised, however, that due to limitation of space it may not be always possible. In such cases, only those samples should be stored at room temperature in which chemical preservative has been added or which do not require preservation at 4 °C.

Samples for BOD, coliform, chlorophyll-a, pesticides and other organics which are likely to volatilise, should never be stored at room temperature.

One important factor which can help to minimise the storage time for samples is sensible long-term planning of the sampling programme. For example, the programme should be planned so that the laboratory does not receive at one time any more samples than it can readily analyse. It is better, therefore, in order to ensure that the analytical results are as accurate as possible, to plan for the laboratory to receive samples in small batches rather than all at one time.

A further factor which needs to be considered in planning the sampling programme is the necessity of avoiding taking too many samples near to weekends and laboratory staff holiday periods as this will increase the time required to analyse the samples and unnecessarily increase storage times.

Table 3.2 on the next page gives maximum permissible storage times for various water quality parameters.

3.3 WORK DISTRIBUTION

The laboratory incharge should maintain a bound register for assignment of work. This register would link the lab. sample number to the analyst who makes specific analyses, such as pH, EC, BOD, etc. An estimate of time needed for performing the analyses may also be entered in the register.

When considering the analysis of a sample for a number of different parameters, it is important that the determinands which are likely to degrade within a short period of time are analysed first. Thus, when a batch of samples is received by the laboratory, the analytical effort needs to be carefully planned to reflect this.

Each laboratory analyst should have his/her own bound register, where all laboratory readings and calculations are to be entered. When more than one sample are to be analysed for the same parameter(s), it is advisable to complete the analysis parameter-wise. This will save time and effort.

When analysis and calculations are completed, the results must be recorded in a register containing data record sheets described in Chapter 5.

Parameter Group	Parameter	Recommended ¹	Required ²
General	Temperature	Immediate	Immediate
	Suspended Solids	7 days	7 days
	Conductivity	28 days	28 days
	pH	Immediate	Immediate
	Dissolved Oxygen ³	8 hours	8 hours
	Dissolved Solids	7 days	7 days
Nutrients	Ammoniacal Nitrogen ³	7 days	28 days
	Total Oxidised Nitrogen ³	---	28 days
	Total Phosphorus	---	---
Organic Matter	Chemical Oxygen Demand ³	7 days	28 days
	Biochemical Oxygen Demand	6 hours	48 hours
Major Ions	Sodium ³	6 months	6 months
	Potassium ³	6 months	6 months
	Calcium ³	6 months	6 months
	Magnesium ³	6 months	6 months
	Carbonates and Bicarbonates ⁴	24 hours	14 days
	Chloride	28 days	28 days
	Sulphate	28 days	28 days
Other Inorganics	Silica	28 days	28 days
	Fluoride	28 days	28 days
	Boron	28 days	6 months
Metals	Cadmium ³	6 months	6 months
	Mercury ³	28 days	28 days
	Zinc ³	6 months	6 months
Organics	Pesticide (Indicator)	7 days	7 days
	Synthetic Detergents	---	---
	Organic Solvents	---	---
	Phenols ³	---	28 days
Microbiological	Total coliforms	8 hours	24 hours ⁴
Biological	Chlorophyll-a	30 days	---

Table 3.2: Maximum permissible storage times for various water quality parameters.

¹ Time limits taken from those recommended in, 'Standard Methods for the Examination of Water and Wastewater', 19th Edition 1995, Eds: Eaton A D, Clesceri L S and Greenberg A E; Published by: APHA, AWWA and WEF

² Required means that unless the parameter is determined within this time period the results will have little validity. Times correspond to limits imposed by the US Environmental Protection Agency for 'regulatory' samples.

³ After recommended pre-treatment (see Field Manual, Vol 6, Chapter 3)

⁴ Time limit stated is equivalent to that given for 'alkalinity' analysis

4 RECOMMENDED ANALYTICAL PROCEDURES

4.1 INTRODUCTION

The analytical procedures given in this chapter are 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 Standard Methods, either the 19th or previous editions.
- 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 in place of the procedure given in the Standard Methods.
- The Standard Methods is an internationally recognised treatise, which is revised and updated every two to five years.

The Standard Methods as presented in this Chapter are conformable to those presented in *Guidelines on Standard Analytical Procedures*, May 1999 (Version 2).

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

4.2 STANDARD ANALYTICAL PROCEDURES

Figure 4.1 shows the heading format for each of the methods. Different methods are listed and identified by a unique name, abbreviation and identification number. This abbreviation or code is also used in the surface water data entry software (SWDES). The version number gives the number of updates that took place since introduction of the first version of a method.

Alk-P	AIKALINITY, PHENOLPHTHALEIN (pH 8.3)
Method:	TITRIMETRIC TO PH=8.3 (PHENOLPHTHALEIN)
ID: 1.30	Version: 2

Figure 4.1 Specimen of header for a Standard Analytical Procedure

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

Apparatus

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

Reagents

- Standard sodium carbonate, approximately 0.05 N: Dry 3 to 5 g sodium carbonate, Na_2CO_3 , at 250°C for 4h and cool in a desiccator. Accurately weigh 2.5 ± 0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.
- Standard H_2SO_4 , approximately 0.1N: Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N 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 the Na_2CO_3 standard (see a.)

B = mL Na_2CO_3 solution taken for standardisation titration

C = mL acid used in standardisation titration

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

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

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

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

Procedure

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

$$\text{Phenolphthalein alkalinity, mg CaCO}_3/\text{L} = \frac{A \times N \times 50000}{\text{mL sample}}$$

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.

Reporting

Phenolphthalein Alkalinity should be reported in units of mg CaCO₃/L, and should include 1 digit after the decimal point, e.g. 26.7 mg CaCO₃/L.

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

Apparatus

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

Reagents

- Standard sodium carbonate, approximately 0.05N: Dry 3 to 5 g sodium carbonate, Na_2CO_3 , at 250 °C for 4 h and cool in a desiccator. Accurately weigh 2.5 ± 0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.
- Standard H_2SO_4 , approximately 0.1N: Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N 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 the Na_2CO_3 standard (see a.)

B = mL Na_2CO_3 solution taken for standardisation titration

C = mL acid used in standardisation titration

- In case potentiometric titration is not possible use bromcresol green indicator to complete the titration.
- Standard sulphuric acid, 0.02N: Dilute the approximate 0.1N solution to 1 L. Calculate volume to be diluted as:

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

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

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

Procedure

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

$$\text{Total alkalinity, mg CaCO}_3/\text{L} = \frac{B \times N \times 50000}{\text{mL sample}}$$

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.

Reporting

Total Alkalinity should be reported in units of mgCaCO₃/L and should include 1 digit after the decimal point, e.g. 42.7 mg CaCO₃/L.

AI	ALUMINUM
Method:	ERIOCHROME CYANINE R SPECTROPHOTOMETRIC
ID: 1.30	Version: 2

Apparatus

- a. Spectrophotometer: For use at 535 nm 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, $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 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, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in water, add 40 mL 1N 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, 1N & 0.1N.

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 1 mL 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 bromocresol 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.

Reporting

Aluminium should be reported in units of mg/L and should include 2 digits after the decimal point e.g. 2.35 mg/L.

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

 **Procedure**

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

Calculation

In case total dissolved solids < 500 mg/L

$$\text{HCO}_3^- \text{ as mg CaCO}_3/\text{L} = \frac{T - 5.0 \times 10^{(\text{pH}-10)}}{1 + 0.94 \times 10^{(\text{pH}-10)}}$$

where: T = total alkalinity as mg CaCO₃/L

The above calculations are based on ionisation constants of carbonic acid at 25 °C 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 CaCO₃/L) as follows:

Alkalinity result	Bicarbonate, mg CaCO₃/L
P = 0	T
P < ½T	T-2P
P = ½T	0
P > ½T	0
P = T	0

Table 4.1: Alkalinity and Bicarbonate

Convert to desired units:

$$\text{HCO}_3^- (\text{mg /L}) = \text{HCO}_3^- (\text{mgCaCO}_3 /\text{L}) \times 1.22$$

Reporting

Bicarbonate should be reported in units of mg/L and should include 1 digit after the decimal point, e.g. 20.9 mg/L.

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

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 $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 1.7 g NH_4Cl in 1 L distilled water.
- b. Magnesium sulphate solution: Dissolve 22.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 L distilled water.
- c. Calcium chloride solution: Dissolve 27.5 g CaCl_2 in 1 L distilled water.
- d. Ferric chloride solution: Dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 1 L distilled water.
- e. Acid and alkali solution: 1N NaOH and 1N 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 1 L 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 mg/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 4.2: 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 3 days.

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:

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

- b. When dilution water is seeded:

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

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 of 198 ± 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.

Reporting

BOD should be reporting in units of mg/L and should include 1 digit after the decimal place e.g. 6.5 mg/L.

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

Apparatus

- a. Spectrophotometer, or photometer with a green filter, for use at 540 nm.
- b. High-silica glass or porcelain evaporating dishes, 100 – 150 mL
- c. Water-bath, set at 55 °C
- d. Glass-stoppered volumetric flasks, 25 – 50 mL capacity.
- e. Ion- exchange column, 1.3 cm diameter, 50 cm length.
- f. Containers, boron free or polyethylene.

Reagents

- a. Stock boron solution: Dissolve 571.6 mg anhydrous boric acid, in distilled water and dilute to 1 L, 1 mL = 100 µg B.
- b. Standard boron solution: Dilute 10 mL stock boron solution to 1 L with distilled water; 1 mL = 1µg B.
- c. Curcumin reagent: Dissolve 40 mg finely ground curcumin and 5 g 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 and 1 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 – 1 mg 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 100 mg /L hardness as CaCO₃. 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 540 nm.

Calculation

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

Reporting

Boron should be reported in units of mg/L and should include 2 digits after the decimal point, e.g. 1.62 mg/L.

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

Reagents

- Sodium hydroxide, NaOH, 1N.
- Murexide (ammonium purpurate) indicator: Mix 200 mg dye with 100 g solid NaCl. Grind to 40 to 50 mesh size.
- 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.
- Standard calcium solution: Weigh 1.000 g anhydrous CaCO₃ in 500 mL flask (primary standard). Add 1 + 1 HCl in small amounts through a small funnel till all CaCO₃ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO₂. Cool and add a few drops of methyl red indicator and adjust to intermediate orange colour by adding 3N NH₄OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 400.8 µg Ca.

Procedure

- 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.
- 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

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

Notes

Although Calcium concentration should be reported in mg/L, Calcium is sometimes also referred to as "Calcium Hardness". Calcium Hardness refers to the amount of Calcium present in terms of mg CaCO₃/L.

The units are related by:

$$\text{mg Ca/L} \times 2.5 = \text{mg CaCO}_3/\text{L}$$

$$(2.5 = 100 \text{ g CaCO}_3/40 \text{ g Ca})$$

Reporting

Calcium should be reported in units of mg/L and should include 1 digit after the decimal point, e.g. 12.3 mg/L.

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

Procedure

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

Calculation

In case total dissolved solids < 500 mg/L:

$$\text{HCO}_3^- \text{ as mg CaCO}_3 = \frac{T - 5.0 \times 10^{(\text{pH}-10)}}{1 + 0.94 \times 10^{(\text{pH}-10)}}$$

$$\text{CO}_3^{2-} \text{ as mg CaCO}_3/\text{L} = 0.94 \times \text{HCO}_3^- \times 10^{(\text{pH}-10)}$$

where: T = total alkalinity (Alk-T) as mg CaCO₃/L

HCO₃⁻ = bicarbonate

The above calculations are based on ionisation constants of carbonic acid at 25 °C 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 phenolphthalein alkalinity, P and total alkalinity, T (both mg CaCO₃/L) as follows:

Alkalinity result	Carbonate, mg CaCO₃/L
P = 0	0
P < ½T	2P
P = ½T	2P
P > ½T	2(T-P)
P = T	0

Table 4.3: Alkalinity and Carbonate

Convert to desired units: CO₃ (mg/L) = CO₃ (mg CaCO₃/L) X 0.6

(because 1 mole = 100 g CaCO₃ = 60 g CO₃)

Reporting

Carbonate should be reported in units of mg CaCO₃/L and should include 1 digit after the decimal point, e.g. 16.6 mg/L.

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

Apparatus

- Reflux flasks, consisting of 250 mL flask with flat bottom and with 24/29 ground glass neck
- 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.
- Hot plate or gas burner having sufficient heating surface.

Reagent

- 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 1 L.
- Sulphuric acid reagent: Add 5.5 g Ag_2SO_4 technical or reagent grade, per kg of conc. H_2SO_4 , keep for a day or two to dissolve.
- Ferriin indicator solution: Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. Commercial preparation may also be available.
- Standard ferrous ammonium sulphate (FAS), titrant, 0.25M: Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water, add 20 mL conc. H_2SO_4 , cool and dilute to 1 L, standardise daily as follows.
- 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 ferriin indicator and titrate with FAS.
- $$\text{Molarity FAS} = \frac{\text{Volume of 0.0417M } K_2Cr_2O_7, \text{ mL}}{\text{Volume of FAS used, mL}} \times 0.25$$
- Mercuric Sulphate, $HgSO_4$, powder
- Potassium hydrogen phthalate (KHP) standard: Lightly crush and dry potassium hydrogen phthalate ($HOOC C_6H_4 COOK$), at 120 °C, cool in desiccator, weigh 425 mg in distilled water and dilute to 1 L. This solution has a theoretical COD of 500 $\mu gO_2/mL$, stable for 3 months in refrigerator.

Procedure

- 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

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

- 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 ₂ SO ₄ with Ag ₂ SO 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

Table 4.4: Sample volumes and quantity of Chemicals

Reporting

COD should be reported in units of mg/L and should include 1 digit after the decimal point, e.g. 9.7 mg/L.

CI	CHLORIDE
Method:	ARGENTOMETRIC TITRATION
ID: 1.33	Version: 1

Reagents

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

where: N = normality of $AgNO_3$
V = mL $AgNO_3$ titrant
B = mL titration for blank
- Standard sodium chloride, 0.0141M (0.0141N): Dissolve 824.0 mg NaCl (dried at 140 °C) in distilled water and dilute to 1000 mL; 1 mL = 500 μg Cl^-
- Aluminium hydroxide suspension: Dissolve 125 g aluminium potassium sulphate or aluminium ammonium sulphate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$, in 1 L distilled water. Warm to 60 °C and add 55 mL concentrated ammonium hydroxide slowly with stirring. Let stand for about 1 h, transfer to a large bottle. Wash precipitate two times or till free of chloride, by successive addition of distilled water, settling and decanting.

Procedure

- 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.
- Add 1 mL K_2CrO_4 indicator solution, titrate with $AgNO_3$ titrant to a pinkish yellow end point.
- Repeat the titration with distilled water blank. A blank of 0.2 to 0.3 mL is usual.

Calculation

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

where: A = mL titration for sample

B = mL titration for blank

N = normality of AgNO_3

Reporting

Chloride should be reported in units of mg/L and should include 0 digits after the decimal place, e.g. 32 mg/L.

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

Apparatus

- a. Spectrophotometer
- b. Cuvettes, with 1, 4, and 10 cm 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 1 g finely powdered MgCO₃ 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 2 h 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 500 g, 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 664 nm. Acidify extract in the cuvette with 0.1 mL 0.1N HCl, gently agitate the acidified extract and read OD at 750 and 665 nm, 90 s 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 1 cm before making calculations.

Calculation

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

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

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 $A \times K$

where: A = absorbance coefficient for chlorophyll-a, at 664 nm = 11.0

K = ratio expressing correction for acidification, 2.43

Reporting

Chlorophyll-a should be reported in units of $\mu\text{g/L}$ and should be 1 digit after the decimal point, e.g. 12.5 $\mu\text{g/L}$.

FCOL-MPN	COLIFORMS, FAECAL
Method:	ELEVATED TEMPERATURE FERMENTATION
ID: 1.6	Version: 1

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	1 L

- 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.

Reporting

Faecal Coliforms should be reported in units of MPN/100 mL with 0 digits after the decimal, e.g. 2300 MPN/100 mL.

Tcol-mpn	COLIFORMS, TOTAL
Method:	STANDARD MULTIPLE TUBE FERMENTATION
ID: 1.7	Version: 1

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 1N sodium hydroxide and dilute to 1 L. 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 g
Sodium chloride, NaCl	5.0 g
Sodium lauryl sulphate	0.1 g
Distilled water	1 L

Add ingredients to water, mix thoroughly and heat to dissolve. pH should be 6.8 ± 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 °C for 15 min. and pipettes in metal containers in steriliser oven at 170 °C for 2 h.
- 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.

Table 4.5: Sample volume and number of tubes to be inoculated for various water sources

- For transferring volumes less than 0.1 mL, prepare serial dilutions. To prepare serial dilutions add 1 mL sample to a dilution tube containing 9 mL dilution water. Mix and transfer 1 mL from the first dilution to second dilution tube to given second dilution and so on. Thus 1 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 °C. After 24 ± 2 h swirl each tube and examine for gas production. If no gas is evident, re-incubate and re-examine at the end of total 48 ± 3 h. 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 ± 0.5 °C. Formation of gas within 48 ± 3 h constitutes a positive confirmed phase.

Calculation

1. When ten tubes of 10 mL each are incubated read from Table 4.6.

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 4.6.

2. When three test dilutions are incubated read from Table 4.7.

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 4.7. For smaller inocula also use Table 4.7 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 4.7 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 4.8 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 4.7. 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 4.6: *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 4.7: 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		953
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	2200
9.	0/5	1/5	0/5	0/5			2	2
10.	-	5/5	3/5	1/5	1/5	-	140	1400

Table 4.8: Examples for reading positive tubes: regular reading of Table 4.6 (no 1), using the formula (no 2 and 5) and dealing with more than three test dilutions (no 3-10).

Reporting

Total Coliforms should be reported in units of MPN/100 mL and should have 0 digits after the decimal point, e.g. 5000 MPN/100 mL.

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

Apparatus

Nessler tubes: Matched, 50 mL, tall form.

Reagents

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

Table 4.9: Working standards

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

Procedure

- 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.
- 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.
- 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

- 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

Reporting

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

Table 4.10: Reporting of results for ranges of colour units

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

Apparatus

- DO sampler, for collection of undisturbed samples from surface waters.
- BOD bottles, 300 mL, narrow mouth, flared lip, with tapered and pointed ground glass stoppers.
- A siphon tube, for laboratory use.

Reagents

- Manganous sulphate solution: Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter and dilute to 1L.
- 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 1L. Add 10 g NaN_3 dissolved in 40 mL distilled water.
- Sulphuric acid, conc
- Starch indicator: Dissolve 2 g laboratory grade soluble starch and 0.2 g salicylic acid as a preservative, in 100 mL hot distilled water.
- Standard sodium thiosulphate titrant, 0.025M (0.025N): Dissolve 6.205 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 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.
- Standard potassium bi-iodate solution, 0.0021M (0.0126N): Dissolve 812.4 mg $\text{KH}(\text{IO}_3)_2$ 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 6N H_2SO_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:

$$M = \frac{20 \times 0.025}{V}$$

where: V = mL of thiosulphate used

Procedure

- Drain any liquid in the flared lip of the BOD bottle containing the sample.
- 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 H₂SO₄ and re-stopper. Mix by inverting several times until dissolution is complete.
- e. Titrate 201 mL with standard Na₂S₂O₃ 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

Reporting

Dissolved oxygen should be reported in units of mg/L and should include 1 digit after the decimal point, e.g. 8.2 mg/L.

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 0.1 mS/m whichever 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, $H_2PtCl_6 \cdot 6H_2O$ and 12 mg lead acetate in 100 mL distilled water. Immerse electrodes in this solution and connect both to the negative terminal of a 1.5 V 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 0.1 $\mu mho/cm$.
- b. Standard potassium chloride solution, KCl, 0.01M, conductivity 1412 $\mu mho/cm$ at 25 °C. Dissolve 745.6 mg 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 0.01 M 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 1412 $\mu mho/cm$ 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 $[0.0191 \times (t-25)+1]$ can be read from Table 4.11.

- 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:

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

where: K_C = the cell constant, 1/cm

C_M = measured conductance of the sample, μmho

t = observed temperature of sample, °C

The value of temperature correction $[0.0191 \times (t-25)+1]$ can be read from Table 4.11.

- 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 $\mu\text{mho/cm}$, use Table 4.12 for conversion of units.

T (°C)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.08	0.09
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	0.934	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	10.55
28	1.057	1.059	1.061	1.063	1.065	1.067	1.069	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	1.448	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	1.194	1.194	1.196	1.198	1.200	1.201	1.203	1.205	1.207

Table 4.11: Value of $[0.0191 \times (T-25) + 1]$ for Temperature Correction of EC measurement

Multiply	by	to obtain
$\mu\text{S/m}$	0.01	$\mu\text{mho/cm}$
mS/cm	10	$\mu\text{mho/cm}$
mS/cm	1000	$\mu\text{mho/cm}$
$\mu\text{S/cm}$	1	$\mu\text{mho/cm}$
mmho/cm	1000	$\mu\text{mho/cm}$

Table 4.12: Conversion table for units of electrical conductivity

Note: 1 S = 1 mho

Reporting

Report electrical conductivity in units of $\mu\text{mho/cm}$ with 0 digits after the decimal point, e.g. 364 $\mu\text{mho/cm}$.

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

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 1000 mL; 1.00 mL = 100 $\mu\text{g F}^-$.
- b. Standard fluoride solution: Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1 mL = 10 $\mu\text{g F}^-$.
- 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 6N NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to the 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.

Reporting

Fluoride should be reported in units of mg/L and should have 2 digits after the decimal point, e.g. 7.46 mg/L.

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

Apparatus

- a. Distillation apparatus: 1 L 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 4.1. Alternative types of distillation apparatus may be used.
- b. Spectrophotometer for use at 570 nm: It must provide a light path of at least 1 cm or a spectrophotometer with a greenish yellow filter (550 to 580 nm).

Reagents

- a. Sulphuric acid, H₂SO₄, conc., reagent grade.
- b. Silver sulphate, Ag₂SO₄, crystals, reagent grade.
- c. Stock fluoride solution: Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1 mL = 100 µg F⁻
- d. Standard fluoride solution: Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1 mL = 10 µg F⁻
- e. SPADNS solution: Dissolve 958 mg 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 133 mg zirconyl chloride octahydrate, ZrOCl₂.8H₂O, 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 5 g NaAsO₂ and dilute to 1 L 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. H₂SO₄ 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 AgSO₄ to the distilling flask at the rate of 5 mg/mg Cl⁻ to avoid Cl⁻ interference. H₂SO₄ 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

Table 4.13: Volumes of Standard Fluoride solution

- 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 570 nm). 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) NaAsO₂ 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

$$\text{mg F}^{-}/\text{L} = \frac{A}{B} \times \frac{1}{R}$$

where: A = µg F⁻ reading from the standard curve.

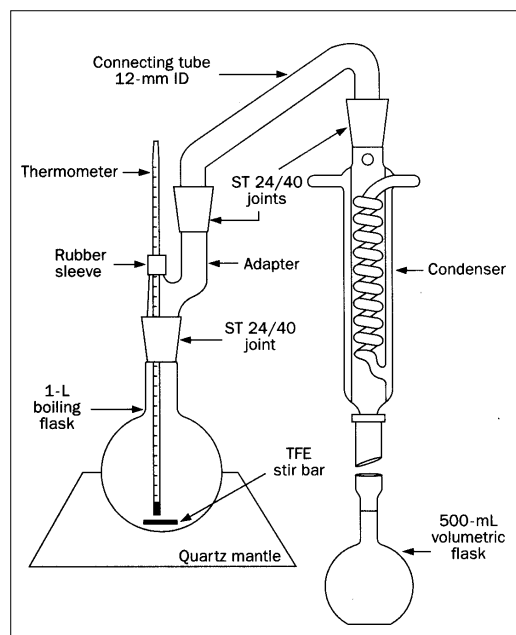
B = volume of diluted or undiluted sample taken for colour development, mL

R = ratio of the volume of sample taken for dilution to the final volume after dilution, when sample is diluted

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 (SO₄²⁻)	200	-

Table 4.14: Concentration of substances causing 0.1mg/L error at 1.0mg F/L in SPADNS method. (+) overestimation, (-) underestimation of actual Fluoride concentration.



*Figure 4.1:
Fluoride Distillation Apparatus*

Reporting

Fluoride should be reported in units of mg/L and should have 2 digits after the decimal point, e.g. 7.46 mg/L.

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

Reagents

- Buffer solution¹: Dissolve 16.9 g NH₄Cl in 143 mL conc. NH₄OH. Add 1.25 g 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.
- Complexing agent: Magnesium salt of 1,2 cyclohexanediaminetetraacetic acid. Add 250 mg per 100 mL sample only if interfering ions are present and sharp end point is not obtained.
- Indicator: Eriochrome Black T sodium salt: Dissolve 0.5 g dye in 100 mL triethanolamine or 2 ethylene glycol monomethyl ether. The salt can also be used in dry powder form by grinding 0.5 g dye with 100 g NaCl.
- Standard EDTA titrant, 0.01M: Weigh 3.723 g di-sodium salt of EDTA, dihydrate, dissolve in distilled water and dilute to 1000 mL. Store in polyethylene bottle.
- Standard Calcium Solution: Weigh 1.000 g anhydrous CaCO₃ in a 500 mL flask. Add 1 + 1 HCl slowly through a funnel till all CaCO₃ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO₂. Cool and add a few drops of methyl red indicator and adjust to the intermediate orange colour by adding 3N NH₄OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 1 mg CaCO₃.

Procedure

- 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 than 15 mL EDTA titrant and complete titration within 5 min after buffer addition.
- 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 CaCO₃ equivalent to 1.00 mL EDTA titrant

Note

- If the Mg salt of EDTA is unavailable (or too expensive) dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (analytical reagent grade) and 780 mg magnesium sulfate (MgSO₄·7H₂O) or 644 mg magnesium chloride (MgCl₂·6H₂O) in 50 mL distilled water. Add this solution to 16.9 g NH₄Cl and 143 mL conc. NH₄OH 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 MgSO₄ or MgCl₂.

Reporting

Total Hardness should be reported in units of mg CaCO₃/L and should have 0 digits after the decimal place, e.g. 280 mg CaCO₃/L.

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

Apparatus

- a. Spectrophotometer, for use at 510 nm, providing a light path of 1 cm 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 NH₂OH.HCl in 100 mL distilled water.
- c. Ammonium acetate buffer solution; dissolve 250 g NH₄C₂H₃O₂ 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 NaC₂H₃O₂·3H₂O in 800 mL water.
- e. Phenanthroline solution; dissolve 100 mg 1,10-Phenanthroline monohydrate, C₁₂H₈N₂·H₂O, 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 H₂SO₄ to 50 mL water and dissolve 1.404 g ferrous ammonium sulphate, Fe(NH₄)₂(SO₄)₂·6H₂O; add 0.1N KMnO₄ 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 = 1.0 µg 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 NH₂OH. 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 NH₄C₂H₃O₂ 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 510 nm.
- 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 $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ 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 1-10 μg iron.

Calculation

Read from the calibration curve and calculate the iron content.

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

Reporting

Iron should be reported in units of mg/L and should have 1 digit after the decimal point, e.g. 6.6 mg/L.

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

Procedure

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

Calculation

$$\text{mg Mg/L} = (\text{TH as mg CaCO}_3\text{/L} - \text{Calcium Hardness as mg CaCO}_3\text{/L}) \times 0.243$$

where: TH = Total Hardness, mg CaCO₃/L

Reporting

Magnesium should be reported in units of mg/L and should include 1 digit after the decimal point, e.g. 12.2 mg/L.

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

Apparatus

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

Reagents

- a. Special reagent: Dissolve 75 g HgSO₄ in 400 mL conc HNO₃ and 200 mL distilled water. Add 200 mL 85% phosphoric acid and 35 mg silver nitrate. Dilute the cooled solution to 1 L.
- b. Ammonium persulphate, (NH₄)₂S₂O₈, solid.
- c. Standard manganese solution, 1.00 mL = 50.0 µg Mn: Dissolve 3.2 g KMnO₄ 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 Na₂C₂O₄ and transfer to 400 mL beakers. To each beaker, add 100 mL distilled water and stir to dissolve.
 - Add 10 mL 1 + 1 H₂SO₄ and rapidly heat to 90 to 95 °C.
 - Titrate rapidly with KMnO₄ to slight pink end point. Do not let temperature fall below 85 °C. If necessary, warm during titration. Run a blank on distilled water and H₂SO₄.
 - Calculate normality:

$$\text{Normality of KMnO}_4 = \frac{\text{gNa}_2\text{C}_2\text{O}_4}{(\text{A} - \text{B}) \times 0.06701}$$
 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. H₂SO₄ and NaHSO₃ solution drop wise, until the permanganate colour disappears. Boil to remove excess SO₂, cool and dilute to 1000 mL. Dilute this solution further with distilled water to measure small amounts of Mn.
- d. Sodium oxalate: Na₂C₂O₄, primary standard, solid.
- e. Sodium bisulphite: Dissolve 10 g NaHSO₃ in 100 mL distilled water.
- f. Hydrogen peroxide, H₂O₂, 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 H₂O₂. Concentrate to 90 mL by boiling or dilute to 90 mL.
- b. Add 1 g (NH₄)₂S₂O₈ and boil for 1 min then cool under the tap. Dilute to 100 mL 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

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

Reporting

Manganese should be reported in units of mg/L and should have 2 digits after the decimal point, e.g. 1.23 mg/L.

NH₃-N	NITROGEN, AMMONIA
Method:	DISTILLATION TITRIMETRIC
ID: 1.14	Version: 2

Apparatus

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

- Ammonia free water: Add 0.1 mL conc. H₂SO₄ to 1 L distilled water, redistill and store in a tightly stoppered glass bottle. Use ammonia free water for preparing reagents, rinsing and dilution.
- Borate buffer: Add 88 mL 0.1N NaOH to 500 mL of 9.5 g Na₂B₄O₇ · 10H₂O/L solution, and dilute to 1 L.
- Indicating boric acid solution: Dissolve 20 g H₃BO₃ in water, add 10 mL mixed indicator solution, dilute to 1 L. Prepare monthly.
- Mixed indicator solution: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.
- Standard sodium carbonate, approximately 0.05N. Dry 3 to 5 g sodium carbonate, Na₂CO₃, at 250 °C for 4h and cool in a desiccator. Accurately weigh 2.5±0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.
- Standard H₂SO₄, approximately 0.1N. Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N Na₂CO₃ 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 Na₂CO₃ weighed into the flask

B = mL Na₂CO₃ solution taken for titration

C = mL acid used

- In case potentiometric titration is not possible use bromcresol green indicator to complete the titration. The indicator is prepared by dissolving 100 g bromcresol green sodium salt in 100 mL distilled water.
- Standard sulphuric acid, 0.02 N. Dilute the approximate 0.1 N solution to 1 L. Calculate volume to be diluted as:

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

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

- j. Dechlorinating agent, dissolve 3.5 g sodium thiosulphate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, in water and dilute to 1 L, prepare fresh. 1 mL reagent in 500 mL sample will remove 1 mg/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

Table 4.15: Sample volume as function of Ammonia Nitrogen in sample

- 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 indicating 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

$$\text{mg NH}_3 - \text{N/L} = \frac{(A - B) \times 280}{\text{mL sample}}$$

where : A = mL H_2SO_4 titrated for sample

B = mL H_2SO_4 titrated for blank

Reporting

Ammonia Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 1.45 mg N/L.

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

Apparatus

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

Reagents

- a. Phenol solution: Mix 11.1 mL liquified phenol ($\geq 89\%$) 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 0.5 g 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 1 L.
- 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 3.819 g anhydrous, NH₄Cl, earlier dried at 100 °C and cooled in desiccator, in ammonia free water and dilute to 1 L; 1 mL = 1 mgN = 1.22 mgNH₃.
- 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 640 nm.

Calculation

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

Reporting

Amونيا Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 1.45 mg N/L.

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

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. H₂SO₄ to 1 L distilled water and redistill. Alternatively, prepare de-ionised water from distilled water using a mixed cation and anion exchange 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 (Na₄EDTA.4H₂O) and stir to dissolve. Cool and dilute to 1000mL.
- d. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH₄Cl (dried at 100 °C) in water, dilute to 1000mL; 1 mL = 1 mg 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 up to the mark. Similarly prepare two more successive dilutions to give serial decimal dilutions of 100, 10, 1.0, and 0.1 mg NH₃-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 10N 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 °C 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 NH₃-N/L concentration on logarithmic scale. The calibration curve should be a straight line with a slope of about 59/decade at 25 °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:

$$\text{mgNH}_3 - \text{N/L} = A \times B \times \frac{100 + D}{100 + C}$$

- where:
- A = dilution factor for the sample, if any,
 - B = concentration of NH₃-N, mg/L, from calibration curve
 - C = volume of alkali added to the standards, mL
 - D = volume of alkali added to the sample

Reporting

Ammonia Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 1.45 mg N/L.

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

Procedure

Carry out analysis for TON and NO₂ as described in method 1.41 and 1.17 respectively.

Calculation

$$\text{NO}_3^- = \text{TON} - \text{NO}_2^-$$

where: TON = measured concentration of total oxidised nitrogen (NO₃⁻+NO₂⁻)
by method 1.41, mg N/L

NO₂⁻ = measured concentration of nitrite by method 1.17, mg NO₂⁻-N/L

NO₃⁻ = concentration NO₃⁻, mg NO₃⁻-N/L

Reporting

Nitrate Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 6.71 mg N/L.

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

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 (KNO₃) in water and dilute to 1000 mL; 1 mL = 100 µg NO₃⁻ - 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 NO₃⁻ - N/L, respectively.
- d. Buffer solution: Dissolve 17.32 g Al₂(SO₄)₃·18H₂O, 3.43 g Ag₂SO₄, 1.28 g H₃BO₃, and 2.52 g sulfamic acid (H₂NSO₃H), 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 NO₃⁻ - 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 NO₃-N/L standards.
- c. Plot on a semilogarithmic graph paper potential measurement of the standards in mV, on arithmetic scale, vs. NO₃⁻ - 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 NO₃⁻-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.

Reporting

Nitrate Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 6.71 mg N/L.

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

Apparatus

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

Reagents

- Nitrate free water: Use re-distilled or de-ionised water to prepare all solutions.
- Stock Nitrate solution: Dissolve 0.7218 g KNO₃, earlier dried in hot air oven at 105 °C overnight and cooled in desiccator, in distilled water and dilute to 1 L. Preserve with 2 mL of CHCl₃/L; 1 mL = 100 µg NO₃⁻-N, stable for 6 months.
- Standard Nitrate Solution: Dilute 100 mL of stock solution to 1000 mL with water, preserve with 2 mL CHCl₃/L; 1 mL = 10 µg NO₃⁻-N, stable for 6 months.
- Hydrochloric acid solution, HCl, 1N: Cautiously add 83 mL conc. HCl to about 850 mL of distilled water while mixing, cool and dilute to 1 L.

Procedure

- Treatment of sample: Add 1 mL HCl to 50 mL clear/filtered sample, mix.
- Preparation of standard curve: Prepare calibration standards in the range of 0-7 mg NO₃⁻-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
NO₃⁻-N, mg/L	0.2	0.4	0.8	1.4	2.0	3.0	4.0	5.0	6.0	7.0

Table 4.16: Volumes of Standard Nitrate Solutions

- Spectrophotometric measurements: Read absorbance or transmittance against re-distilled water set at zero absorbance or 100 % transmittance. Use a wavelength of 220 nm to obtain NO₃⁻ reading and a wavelength of 275 nm to determine interference due to dissolved organic matter
- If reading at 275 nm is more than 10% of the reading at 220 nm, do not use this method (use method 1.42 instead).

Calculation

For sample and standards, subtract 2 times the absorbance reading at 275 nm, from the reading at 220 nm 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.

Reporting

Nitrate Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g. 6.71 mg N/L.

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

Apparatus

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

Reagents

- Colour reagent: To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulphanilamide. After dissolving add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for one month when stored in dark bottle in refrigerator.
- Sodium oxalate, 0.025M (0.05N): Dissolve 3.350 g Na₂C₂O₄ primary standard grade, in water and dilute to 1000 mL.
- Stock nitrite: Dissolve 1.232 g NaNO₂ in water and dilute to 1000 mL; 1.00 mL = 250 µgN. Preserve with 1 mL CHCl₃. Standardise by pipetting, in order, 50 mL 0.01M KMnO₄, 5 mL conc H₂SO₄ and 50.00 mL stock NO₂⁻ 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) KMnO₄ 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 NO₂⁻ - N/mL in stock solution

B = mL total KMnO₄ used

C = normality of KMnO₄

D = total mL oxalate added

E = normality of oxalate

F = mL stock nitrite taken for titration

- 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 = 50.0 µg NO₂⁻-N. Prepare daily.
- Standard nitrite solution: Dilute 10 mL intermediate NO₂⁻ solution to 1000 mL with water; 1.00 mL = 0.500 µg NO₂⁻-N. Prepare daily.
- Standard potassium permanganate titrant, 0.01M (0.05N): Dissolve 1.6 g KMnO₄ in 1 L distilled water. Allow ageing for 1 week then decant supernatant. Standardise this solution frequently as follows:

- g. Weigh to nearest 0.1 mg several 100 to 200 mg samples of anhydrous sodium oxalate in beakers. To each beaker add 100 mL distilled water, 10 mL 1 + 1 H₂SO₄ 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 + H₂SO₄.

$$\text{normality KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(\text{A} - \text{B}) \times 0.33505}$$

where: A = mL titrant for sample

B = mL titrant for blank

Average the result of several titrations.

Procedure

- Add 2 mL colour reagent to 50 mL sample, or to a portion diluted to 50 mL, and mix.
- Measure absorbance at 543 nm. Wait between 10 min and 2 h after addition of colour reagent before measurement
- 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.

Reporting

Report result as mg N/L with 1 digit after the decimal point, e.g. 0.9 mgN/L.

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. H₂SO₄ to 1 L 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, (9.5 g Na₂B₄O₇·10H₂O/L) and dilute to 1 L.
- c. NaOH, 6N
- d. Dechlorinating agent, dissolve 3.5 g sodium thiosulphate, Na₂S₂O₃·5H₂O, in water and dilute to 1 L, prepare fresh. 1 mL reagent in 500 mL sample will remove 1mg/L chlorine.
- e. Neutralising agents: NaOH, 1N, H₂SO₄, 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 20 g H₃BO₃ in water, add 10 mL mixed indicator solution and dilute to 1 L, prepare every month.
- h. Sulphuric acid, 0.04N: Dilute 1 mL conc H₂SO₄ to 1 L.
- i. Standard sodium carbonate, approximately 0.05N. Dry 3 to 5 g sodium carbonate, Na₂CO₃, at 250 °C for 4h and cool in a desiccator. Accurately weigh 2.5 ± 0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.
- j. Standard H₂SO₄, approximately 0.1N. Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N Na₂CO₃ 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 Na₂CO₃ weighed into the flask
 B = mL Na₂CO₃ 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 100 g bromcresol green sodium salt in 100 mL distilled water.
- l. Standard sulphuric acid, 0.02N. Dilute the approximate 0.1N solution to 1 L. Calculate volume to be diluted as:
- $$\text{mL volume} = \frac{20}{N}$$
- where: N = exact normality of the approximate 0.1N solution.
- m. Digestion reagent: Dissolve 134g K₂SO₄ and 7.3g CuSO₄ in about 800 mL water. Slowly add 134 mL conc. H₂SO₄ with mixing, cool to room temperature, dilute to 1 L with water.
- n. Sodium hydroxide thiosulphate reagent. Dissolve 500 g NaOH and 25 g Na₂S₂O₃·5H₂O in water and dilute to 1 L.
- 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

Table 4.17: Sample size as function of Organic Nitrogen Sample

- 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 6N 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 0.02N H₂SO₄ titrant until indicator turns pale lavender.

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

Calculation

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

where: A = mL of H₂SO₄ titrated for sample

B = mL of H₂SO₄ titrated for blank

Reporting

Report results as mg N/L, with 1 digit after the decimal point, e.g. 6.8 mg N/L.

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

Apparatus

- Reduction column: Columns can be constructed by modification of 100 mL volumetric pipette (see Figure 4.2). Metering valve is recommended to control flow rate.
- Spectrophotometer for use at 543 nm or filter photometer with green filter, maximum transmittance near 540 nm, providing 1 cm light path or longer.

Reagents

- Nitrate free water: Use as reagent blank. The NO₃-N concentration should not exceed 0.01 mg/L. Use nitrate free water for all solutions and dilutions.
- Copper-cadmium granules: Wash 25 g 20- to 100-mesh Cd granules with 6N HCl and rinse with water. Swirl granules with 100 mL 2% CuSO₄ 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.
- Ammonium chloride-EDTA solution: Dissolve 13 g NH₄Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust pH to 8.5 with conc. NH₄OH and dilute to 1 L.
- Dilute Ammonium chloride-EDTA solution: Dilute 300 mL Ammonium chloride-EDTA solution to 500 mL with water.
- Hydrochloric acid, HCl, 6N.
- Copper sulphate solution, 2%: Dissolve 20 g CuSO₄.5H₂O in 500 mL water and dilute to 1 L
- 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 1 L with water. Solution is stable for one month when stored in dark bottle in refrigerator.
- Stock nitrite: Dissolve 1.232 g NaNO₂ in water and dilute to 1000 mL; 1.00 mL = 250 µgN. Preserve with 1 mL CHCl₃. Standardise by pipetting, in order, 50 mL 0.01M KMnO₄, 5 mL conc. H₂SO₄ and 50.00 mL stock NO₂⁻ 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) KMnO₄ 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 NO₂⁻ - N/mL in stock solution

B = mL total KMnO₄ used

C = normality of KMnO₄

- 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 = 50.0 µg NO₂⁻-N. Prepare daily.
- j. Stock Nitrate solution: Dissolve 0.7218 g KNO₃, earlier dried in hot air oven at 105 °C overnight and cooled in desiccator, in distilled water and dilute to 1 L. Preserve with 2 mL of CHCl₃/L; 1 mL = 100 µg NO₃⁻-N, stable for 6 months.
- k. Intermediate Standard Nitrate Solution: Dilute 100 mL of stock solution to 1000 mL with water, preserve with 2 mL CHCl₃/L; 1 mL = 10 µg NO₃⁻-N, stable for 6 months.
- l. Working Nitrite solution: Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite free water; 1 mL = 5 µg NO₂⁻-N.

Procedure

- 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 above granules. Wash the column with 200 mL NH₄Cl-EDTA solution. Activate the column by flushing at least 100 mL of a mixture of 25% 1.0 mg NO₃⁻-N/L standard and 75% NH₄Cl-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 µm membrane filter. Adjust 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 up to less than 4 mg/L NO₃⁻-N/L) and mix with 75 mL NH₄Cl-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 in between different samples is not needed. If columns are not used for more than several hours flush with 50 mL NH₄Cl-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 543 nm: Wait between 10 min and 2 h after addition of colour reagent before measurement. Measure against a distilled nitrate free water reagent blank. If NO₃⁻ 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 NO₃⁻-N solution to prepare standards in the range of 0.05 to 1.0 mg NO₃⁻-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 (NO₂⁻) solution, compare to a reduced nitrate (NO₃⁻) standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described above when reduction efficiency falls below 75%.

Calculation

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.

Reporting

Report as milligrams oxidised N per litre (mg N/L), with 1 digit after the decimal point, e.g. 2.3 mg N/L.

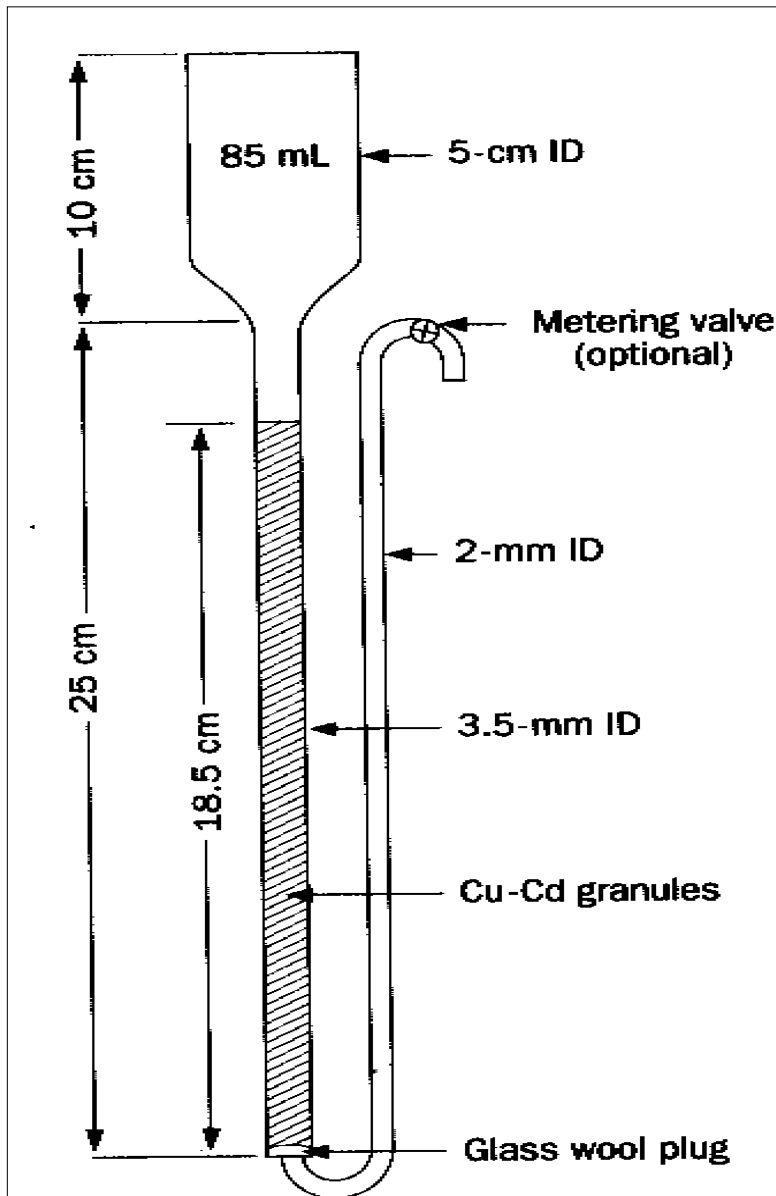


Figure 4.2: Reduction column

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

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

Apparatus

- pH meter with temperature compensating device, accurate and reproducible to 0.1 pH unit with a range of 0 to 14.
- 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.
- Glass electrode. Follow manufacturer's instructions on use and care of electrode.

Reagents

- Potassium hydrogen phthalate buffer, 0.05M, pH 4.00: Dissolve 10.12 g $\text{KHC}_8\text{H}_4\text{O}_4$ (potassium hydrogen phthalate) in 1000 mL freshly boiled and cooled distilled water
- 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
- 0.01M sodium borate decahydrate (borax buffer), pH = 9.18: Dissolve 3.80 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in 1000 mL freshly boiled and cooled distilled water.
- Store buffer solutions in polyethylene bottles. Replace buffer solutions every 4 weeks.

Procedure

- 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.
- 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.
- 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.
- 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.
- Stir the sample gently while measuring pH to insure homogeneity.

Reporting

Report results in pH units, with 1 digit after the decimal point, e.g. 7.6.

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

Apparatus

- a. Spectrophotometer with infrared phototube for use at 880 nm or filter photometer, equipped with a red filter.
- b. Acid washed glassware, use dilute HCl and rinse with distilled water.

Reagents

- a. Sulphuric acid, H₂SO₄, 5N: Dilute 70 mL conc. H₂SO₄ to 500 mL with distilled water.
- b. Potassium antimonyl tartrate solution: Dissolve 1.3715 g K(SbO)C₄H₄O₆.1/2 H₂O in 400 mL distilled water and dilute to 500 mL, store in glass-stoppered bottle.
- c. Ammonium molybdate solution: Dissolve 20 g (NH₄)₆ Mo₇O₂₄.4H₂O in 500 mL distilled water, store in a glass stoppered bottle.
- d. Ascorbic acid, 0.1M: Dissolve 1.76 g ascorbic acid in 100 mL distilled water, keep at 4 °C, use within a week.
- e. Combined reagents: Mix 50 mL 5N, H₂SO₄, 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 219.5 mg anhydrous KH₂PO₄ in distilled water and dilute to 1 L; 1 mL = 50 µg PO₄³⁻ - P.
- g. Standard phosphate solution: Dilute 50 mL stock solution to 1 L with distilled water; 1 mL = 2.5 µg 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 5N H₂SO₄. 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 880 nm. 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

$$\text{o - PO}_4 \text{ as mg P/L} = \frac{\text{mg P}_{\text{from the calibration curve}} \times 1000}{\text{mL sample}}$$

Reporting

Report results in mg P/L, with 3 digits after the decimal point, e.g. 1.452 mg P/L.

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

Apparatus

- a. Hot plate.
- b. Spectrophotometer with infrared phototube for use at 880 nm 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, H₂SO₄ 10N: Carefully add 300 mL conc H₂SO₄ to approximately 600 mL distilled water and dilute to 1 L.
- c. Persulphate: (NH₄)₂S₂O₈ or K₂S₂O₈, solid
- d. Sulphuric acid, H₂SO₄, 5N: Dilute 70 mL conc. H₂SO₄ to 500 mL with distilled water.
- e. Potassium antimonyl tartrate solution: Dissolve 1.3715 g K(SbO)C₄H₄O₆·1/2 H₂O in 400 mL distilled water and dilute to 500 mL, store in glass-stoppered bottle.
- f. Ammonium molybdate solution: Dissolve 20 g (NH₄)₆ Mo₇O₂₄·4H₂O in 500 mL distilled water, store in a glass stoppered bottle.
- g. Ascorbic acid, 0.1M: Dissolve 1.76 g ascorbic acid in 100 mL distilled water, keep at 4 °C, use within a week.
- h. Combined reagents: Mix 50 mL 5N, H₂SO₄, 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 KH₂PO₄ in distilled water and dilute to 1 L; 1 mL = 50µg PO₄³⁻ - P.
- j. Standard phosphate solution: Dilute 50 mL stock solution to 1 L with distilled water; 1 mL = 2.5 µg P.

Procedure

- a. To 50 mL portion of thoroughly mixed sample add one drop phenolphthalein indicator solution. If a red colour develops, add 10N H₂SO₄ to just discharge colour. Then add 1 mL 10N H₂SO₄ and either 0.4 g (NH₄)₂S₂O₈ or 0.5 g K₂S₂O₈.
- 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 colourimetric 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 5N H₂SO₄. 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 880 nm. 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.

Calculation

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

Reporting

Report results as mg P/L with 3 digits after the decimal point, e.g. 2.485 mg P/L.

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

Apparatus

- Flame photometer, direct reading type.
- Glassware, rinse with 1 + 15 HNO₃, followed by de-ionised distilled water.
- Plastic bottles, to store all solutions

Reagents

- Stock potassium solution, weigh 1.907 g KCl, dried at 110 °C and cooled in desiccator, transfer to 1 L volumetric flask and make to 1 L with water; 1 mL = 1.00 mg K.
- Intermediate potassium solution, dilute 10mL stock potassium solution with water to 100 mL; 1mL = 0.1 mg K, prepare calibration curve in the range of 1 to 10 mg/L
- Standard potassium solution: Dilute 10mL intermediate solution with water to 100 mL, 1 mL = 10 µg K, prepare calibration curve in the range of 0.1 to 1 mg/L.

Procedure

- 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.
- 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 potassium concentration of the sample, or diluted sample, from the curve.

Calculation

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

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

Reporting

Report results for Potassium as mg/L, with 1 digit after the decimal point, e.g. 13.9 mg/L.

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

Apparatus

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

Reagents

- a. Store all reagents in plastic containers.
- b. Sulphuric acid, H₂SO₄, 1N.
- c. Hydrochloric acid, HCl, 1 + 1
- d. Ammonium molybdate reagent: Dissolve 10 g (NH₄)₆Mo₇O₂₄·4H₂O 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 NH₄OH or NaOH and store in polyethylene bottle to stabilise.
- e. Oxalic acid solution: Dissolve 7.5 g H₂C₂O₄·H₂O in distilled water and dilute to 100 mL.
- f. Stock silica solution: Dissolve 313.0 mg sodium hexafluorosilicate, Na₂SiF₆, in 1000 mL distilled water; 1 ml = 0.1 mg SiO₂.
- g. Silica standard working solution: Dilute 100 mL stock solution to 1000mL; 1 mL = 10 µg SiO₂.
- h. Reducing agent: Dissolve 500 mg 1-amino-2-naphthol-4-sulphonic acid and 1 g Na₂SO₃ in 50 mL distilled water, with gentle warming if necessary; add this to a solution of 30 g NaHSO₃ 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.

Procedure

- 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.

Calculation

Read silica content of sample from the calibration curve.

Note

Standard Methods recommends use of sodium metasilicate nonahydrate, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, 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.

Reporting

Report results for silicate in units of mgSiO_2/L , with 2 digits after the decimal point, e.g. 1.23 $\text{mg SiO}_2/\text{L}$.

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

Apparatus

- a. Flame photometer, direct reading type.
- b. Glassware, rinse with 1 + 15 HNO₃, followed by de-ionised distilled water.
- c. Plastic bottles, to store all solutions.

Reagents

- a. Stock sodium solution, weigh 2.542 g NaCl, dried at 140 °C and cooled in desiccator, transfer to 1 L volumetric flask and make to 1 L with water; 1 mL = 1.00 mg Na.
- b. Intermediate sodium solution, dilute 10 mL stock sodium solution with water to 100 mL; 1 mL = 0.1 mg Na, prepare calibration curve in the range of 1 to 10 mg/L
- c. Standard sodium solution: Dilute 10 mL intermediate solution with water to 100 mL, 1 mL = 10 µg Na, prepare calibration curve in the range of 0.1 to 1 mg/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

$$\text{mg Na / L} = \text{mg Na / L}_{\text{from the calibration curve}} \times \text{Dilution}$$

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

Reporting

Reporting results for Sodium in units of mg/L, with no digits after the decimal point, e.g. 34 mg/L.

SS	SOLIDS, SUSPENDED
Method:	GRAVIMETRIC AFTER FILTRATION
ID: 1.24	Version: 1

Apparatus

- Glass-fibre filter disk, Whatman grade 934 AH, Gelman type A/E, Millipore type AP40 or equivalent, diameter 2.2 to 12.5 cm.
- Filtration apparatus, Membrane filter funnel or Gooch crucible with adapter and suction flask of sufficient capacity for sample size selected
- Drying oven, 104 ± 1 °C
- Analytical balance capable of weighing up to 0.1 mg.
- Aluminium weighing dishes.

Procedure

- Wash filter paper by putting it on filtration assembly and filtering 3 successive 20 mL portions of distilled water.
- Place filter in an aluminium dish and dry in oven at 104 ± 1 °C for 1 h. If a Gooch crucible is used, dry filter and crucible combination together. Cool in desiccator to balance temperature and weigh.
- Assemble filtration apparatus with the washed, dried and weighed filter paper. Wet filter paper with a small amount of distilled water to seat it.
- 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.
- Wash with 3 successive 10 mL volumes of distilled water. Continue suction for about 3 min after filtration is complete.
- Carefully remove filter and transfer to the aluminium-weighing dish. Dry, cool and weigh as in b above.

Calculation

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

where: A = weight of filter + dried residue, mg, and

B = weight of filter, mg

Reporting

Report results of suspended solids in units of mg/L, with no digits after the decimal point, e.g. 22 mg/L.

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

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

Not required

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 200 mg 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

Reporting

Report results of Total Solids in units of mg/L, with no digits after the decimal point, e.g. 131 mg/L.

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

Procedure

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

Calculation

$$\text{TDS} = \text{TS} - \text{SS}$$

where: TDS = Solids, Total Dissolved, mg/L

TS = Solids, Total, mg/L

SS = Solids, 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.

Reporting

Report results for TDS in units of mg/L, with no digits after the decimal point, e.g. 105 mg/L.

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

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 AP40 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 °C 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 °C. 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 °C below boiling point and raised to 104 °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.

Reporting

Report results for TDS in units of mg/L, with no digits after the decimal point, e.g. 105 mg/L.

SO ₄	SULPHATE
Method:	NEPHELOMETRY
ID: 1.26	Version: 2

Apparatus

- Nephelometric turbidity meter with sample cells. Alternatively a spectrophotometer for use at 420 nm with a light path of 2.5 to 10 cm
- Magnetic stirrer
- Timer with indication of seconds

Reagents

- Buffer solution A: Dissolve 30 g magnesium chloride, MgCl₂·6H₂O, 5 g sodium acetate, CH₃COONa·3H₂O, 1 g potassium nitrate, KNO₃, and 20 mL acetic acid CH₃COOH (99%) in 500 mL distilled water and make up to 1000 mL.
- Buffer solution B: Only required if sample SO₄²⁻ concentration is less than 10 mg/L. Prepare as buffer solution A and add 0.111 g sodium sulphate, Na₂SO₄.
- Barium chloride, BaCl₂, crystals, 20 to 30 mesh
- Standard sulphate solution: Dilute 10.4 mL standard 0.02N H₂SO₄ in to 100 mL (1.00 mL = 100 µg SO₄²⁻).
- Standard sodium carbonate, approximately 0.05N. Dry 3 to 5 g sodium carbonate, Na₂CO₃, at 250 °C for 4 h and cool in a desiccator. Accurately weigh 2.5 ± 0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.
- Standard H₂SO₄, approximately 0.1N. Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N Na₂CO₃ 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 Na₂CO₃ weighed into the 1-L flask for Na₂CO₃ standard (see e.)

B = mL Na₂CO₃ solution taken for standardisation titration

C = mL acid used in standardisation titration

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

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

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

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

Procedure

- Standardise nephelometer following manufacturer's instructions.
- Measure the turbidity of sample-blank, a sample in which no BaCl₂ is added.
- 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 BaCl₂ crystals. Stir for 60 ± 2 s.
- Measure turbidity of the sample at 5 ± 0.5 min after stirring ended
- Prepare SO₄²⁻ standards at 5 mg/L increments in the range of 0- to 40 mg/L SO₄²⁻ 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

Table 4.17: SO₄²⁻ standards at 5 mg/L increments

- Develop BaSO₄ turbidity for the standards as above.
- Determine turbidity of the standards using procedure as above and draw calibration curve between turbidity and SO₄²⁻ concentration, mg/L.
- In case of buffer solution B is used for samples containing less than 10 mg/L SO₄²⁻, 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 SO₄²⁻ 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/mL sample volume.

In case buffer solution B is used, for samples containing less than 10 mg/L sulphate, calculate SO₄²⁻ as follows. Read SO₄²⁻ concentration in the treated sample from the calibration curve after subtracting the turbidity of sample-blank from the turbidity of the treated sample. Subsequently read SO₄²⁻ concentration for the reagents from the turbidity value of the reagent-blank (see procedure 'h') from the calibration curve. Report the corrected SO₄²⁻ concentration in the sample after subtracting the reagent-blank SO₄²⁻ concentration from the sample SO₄²⁻ concentration.

Reporting

Report results for Sulphate in units of mg/L with no digits after the decimal point, e.g. 37 mg/L.

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

Apparatus

Mercury thermometer having a scale marked for every 0.1 °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.

Reporting

Report results for temperature in units of degrees Celsius, with 1 digit after the decimal point, e.g. 21.5 °C.

TURB	TURBIDITY
Method:	NEPHELOMETRIC
ID: 1.28	Version: 1

Apparatus

- a. Nephelometric turbidity meter with sample cells

Reagents

- a. Solution I: Dissolve 1.000 g hydrazine sulphate, $(\text{NH}_2)_2\text{H}_2\text{SO}_4$ in distilled water and dilute to 100 mL in a volumetric flask.
- b. Solution II: Dissolve 10.00 g hexamethylenetetramine, $(\text{CH}_2)_6\text{N}_4$, 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.

Reporting

Report results for turbidity in NTU units, with 1 digit after the decimal point, e.g. 65.7 NTU.

5 ANALYSIS RESULTS

All analysis results should be transferred from the personal laboratory journal of the chemist performing the analysis to the data record and validation register, from where it is entered into the Surface Water Data Entry System (SWDES).

This chapter addresses some important topics on recording information associated with the analysis results.

5.1 METHOD OF ANALYSIS

In course of time the preferred or standard methods of analysis may change. Along with a change of method the analytical results for a variable analysed in a sample may change. Often methods change because newer methods perform better (lower detection limit, less interference with other constituents or a better range of detection). It is therefore important that each laboratory keeps a record of the analytical method it is using. The SWDES contains a list of, common, analytical methods for each parameter and the actual method being used by a laboratory must be selected. If the employed method is not present in the software, the laboratory should keep a record of all necessary details of the method and can add these details to the software.

5.2 AQC

Results of Analytical Quality Control, such as repetitive analysis of standard solutions should not be entered in to the SWDES. The AQC records should be kept in separately. These should be available in the laboratory, preferably in a graphical form (Shewhart Charts).

5.3 NUMBER OF DIGITS IS DEPENDENT ON ACCURACY OF ANALYSIS

To avoid ambiguity in reporting results or in presenting directions for a procedure, it is the custom to use significant figures only. In a significant figure all digits are expected to be known definitely, except the last digit, which may be in doubt. A discussion of significant digits, rules for rounding off and estimating the significant digits in the result of arithmetic operations is given in the Design Manual, Volume 7, Chapter 2. These must be followed in reporting the result of analyses.

5.4 WARNING LEVELS

To avoid entry of incorrect data, which are obviously due to some gross mistake, either in sampling or analysis, the possible range for the values of parameters have been provided in the SWDES. The range is based on prevailing conditions in Indian surface waters. The lower bound is given by the Level of Detection (LOD) of the analysis method used. The data entry software will not accept the out of range values. However, if it is found that the reported values are indeed probable, the limits of the range can be changed.

The software also has provision for setting up user defined 'Warning' limits for a parameter, which reflect the quality of the water. For example, limits may be set to identify the expected range of values in a region. If the reported value exceeds the prescribed limit, the data can be entered but it is flagged.

6 DATA RECORD AND VALIDATION

Figure 6.1 shows the formats for the data record and validation. The first three columns of the table for data record contain the same information as that in the sample receipt register, which will identify the sample. Columns 4 to 9 should be filled in on the basis of the information given in the sample identification form submitted by the field staff when the sample was received by the laboratory. Other columns will contain the results of the analysis. At the time of entering the values for the various parameters, particular attention should be paid to the units, significant numbers and the decimal points.

The lower table is used for data validation. The validation will be done by the SWDES also. However, it is recommended that the validation is done by the laboratory staff manually also, immediately after the analysis is completed. This will allow immediate detection of any gross mistake or an unusual value. In such a case, if the sample is still available, the analysis could be repeated. Columns 37 to 40 and 42 to 46 give the concentrations of cations and anions in meq / L, respectively. Columns 41 and 47 give their totals. Columns 48 to 52 give various checks and column 53 gives the validation criteria.

Data record Laboratory / organisation Laboratory code

Lab sample No	Station code	Date of collection	Field determinations						General				Nutrients			Org matter		Alkalinity		Hardness		Major ions							Other inorganics			Coliforms		Biol		
			pH	EC, μ mho/cm	DO, mg/L	Temp, $^{\circ}$ C	Colour, code	Odour, code	pH	EC, μ mho/cm	TDS, mg/L	TSS, mg/L	NH ₃ , mg N/L	NO ₂ +NO ₃ , mg N/L	Total P, mg/L	BOD, mg/L	COD, mg/L	Phen, mg CaCO ₃ /L	Total, mg CaCO ₃ /L	Total, mg CaCO ₃ /L	Ca ⁺⁺ , mg CaCO ₃ /L	Ca ⁺⁺ , mg/L	Mg ⁺⁺ , mg/L	Na ⁺ , mg/L	K ⁺ , mg/L	Cl ⁻ , mg/L	SO ₄ ⁻ , mg/L	CO ₃ ⁻ , mg/L	HCO ₃ ⁻ , mg/L	Si, mg/L	F ⁻ , mg/L	B, mg/L	Total, MPN/100 mL	Faecal, MPN/100mL	Chlorophyll-A, μ g/L	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	

Data validation

Lab sample No	Station code	Cations					Anions						Ion balance		EC bal	Carbon bal	CO ₃ ⁻ bal	Verification criteria	Checked by	Remarks
		Ca ⁺⁺ , meq/L	Mg ⁺⁺ , meq/L	Na ⁺ , meq/L	K ⁺ , meq/L	Total cations	Cl ⁻ , meq/L	SO ₄ ⁻ , meq/L	CO ₃ ⁻ , meq/L	HCO ₃ ⁻ , meq/L	NO ₂ +NO ₃ , meq/L	Total anions	$\frac{\{(41)-(47)\}}{\{(41)+(47)\}}$	$\frac{(39)}{(42)}$	$\frac{(12)}{(11)}$	$\frac{(18)}{(17)}$	If (10) < 8.3, is (19)=0 ?	(53)	(54)	(55)
(1)	(2)	(37)	(38)	(39)	(40)	(41)	(42)	(43)	(44)	(45)	(46)	(47)	(48)	(49)	(50)	(51)	(52)	(53)	(54)	(55)

Figure 6.1: Data record and validation register