Standard Guide for Water and Waste Water Quality Measurement Methods

2021

" Part :2"

Prepared by:

Steering Committee

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(I) Physical Parameters and Aggregate Properties

(I.1) pH Value
(Electrometry)

Principle:

The pH of a water sample is determined by the measurement of a voltage produced between an electrode responsive to hydrogen ions (glass electrode) and a reference electrode (usually calomel electrode or silver/silver chloride electrode) when both are immersed in the sample. A difference of 1 pH unit produces a potential change of 58.16 mV at 25°C. The pH-meter should be calibrated to read the pH-value directly.

Equipment:

1. pH-Meter (accuracy 0.1 pH units).Combined glass/calomel, or glass/silver- silver chloride electrode.
2. Magnetic stirrer.
3. Teflon-coated stirring bar.

Reagents:

1. Standard pH buffer solutions (pH 4, 7 and 9).
2. Bi-distilled water.
3. Soft tissue paper.

Procedure:

1. Rinse the electrode(s) with bi-distilled water and dry by gentile wiping with a soft tissue paper. This should be done each time before applying the electrode to a new buffer solution or sample.
2. If manual temperature compensation is provided, adjust it to the temperature of the buffer or sample to be measured.
3. Calibrate the instrument by immersing the rinsed electrode(s) in a buffer solution of pH 7 and set the meter to read 7.00 by adjusting the asymmetry control.
4. Rinse the electrode and repeat using a buffer solution of pH 4 or pH 9. Select the possibility which is coming closest to the pH of the sample to be measured.
5. Adjust the reading to the appropriate number by the slope control and discard the portions of the buffer used in the calibration.
6. Immerse the rinsed combination glass electrode and temperature corrected electrode in the unknown sample solution and take a reading after 30 seconds.
7. Express the results to the nearest 0.1 pH unit.
8. The temperature of the sample at the time of pH measurement should be indicated.

Precautions:

1. Before making any measurement, rinse the electrodes with bi-distilled water and dry with tissue paper.
2. Always adjust the instrument to the temperature of the sample.
3. Ensure an adequate level of filling solution in the reference electrode.

4. For storage, always keep the electrode(s) in bi-distilled water and never allow them to dry out.
5. Preferably the pH of any sample should be established at the sampling site, or as soon as possible after taking the sample.
6. No chemical preservation of the sample is permissible to samples subjected for pH measurements.
7. In case high salinity, a sodium ion error at and above pH 10 has to be compensated.

Interferences:

1. Oil and grease may coat the electrodes, giving sluggish results. Gently wipe clean to remove interference.
2. pH is temperature-dependent.
3. Allow buffers and samples to have the same time to warm up to room temperature before measurements.

References:

– USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 150.1).
– "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association, (APHA) Washington 2017(Method 4500-H).

(I.2) Temperature

(Thermometry)

Principle:

Temperature measurements are made with any good mercury-filled Celsius thermometer. As a minimum, the thermometer should have a scale marked for every 0.1 °C, with markings etched on the capillary glass. The thermometer should have a minimal thermal capacity to permit rapid equilibration.

Equipment:

Mercury thermometer, thermos-phone, or thermistor.

Procedure:

1. Record readings with the thermometer immersed in water long enough to permit complete equilibration.
2. Report results to the nearest 0.1 °C or 1.0 °C, depending on the need.
3. Depth temperature required for limnlogical studies may be measured with a reversing thermometer.

Calculation:

Correct the readings of reversing thermometers for changes due to differences between temperature at reversal and temperature at time of readings.

Calculate as follows:

$T = [(T_1-t)\ (T1+V_o)] / K \times [1 + [(T_1-t)\ (T_1+Vo)] /K] + L$

Where:

T = correction to be added algebraically to uncorrected reading.

T_1 = uncorrected reading at reversal.

t = temperature at which thermometer is read.

V_o = volume of small bulb end of capillary up to 0°C.

K = constant depending on relative thermal expansion of mercury and glass (usual value of K= 6100).

L = calibration correction of thermometer depending on T_1.

If series observations are made, it is convenient to prepare graphs for a thermometer to obtain T from any values of T_1 and t.

Precautions:

1. Periodical check of the thermometer against a precision thermometer should be done.
2. The thermometer should be certified by the National Institute of Standards and Technology (NIST).
3. Metals case is used to prevent breakage during field operation.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 170.1).

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 2550- B).

(I.3) Electrical Conductivity

(Electrometry)

Principle:

Conductivity is the ability of an aqueous solution to carry an electrical current. This ability depends upon the presence of ions; on their total concentration, mobility, valence; and temperature. A conductivity cell consisting of two platinum electrodes is calibrated using a standard KCl solution and used for measurements.The method is suitable for measuring the conductivity over the ranges of 0-199.9 mS/cm. Specific conductance, µS at 25°C, and conductivity values give a rough estimate of the electrolyte dissolved solids.

Equipment:

1. Conductivity meter equipped with conductivity cell.
2. Small beaker.

Reagents:

1. Conductivity water: Any of several methods can be used to prepare reagent water-grade. The conductivity should be small compared to the value being measured.
2. Standard potassium chloride solution, KCl (0.010 M): Dissolve (0.7456 g) of anhydrous KCl in conductivity water and dilute to 1000 ml in a class (A) volumetric flask at 25°C. This standard reference solution has a conductivity of 1412 µS/cm.
3. Sample quality control preparation (QC1) 0.02 M KCl: Dissolve (1.4912 g) of anhydrous potassium chloride (KCl) in conductivity water and dilute to 1000 ml in a class (A) volumetric flask at 25 °C. This solution has a conductivity of 2760 µS/cm.
4. The quality control solution must be measured before analysis and after every 20 samples when samples at high range (saline water) are measured. Record the data of QC and make control chart.
5. Sample quality control (QC2) 84 µS/cm: Ready-made 84 µS/cm standard was used as QC2 when samples at low rang (fresh and surface water) are to be measured.

Procedure:

Unless otherwise specified in this method, clean all glassware as in the introduction for the washing of glassware.

A. Calibration:

1. Change the distilled water in the probe storage beaker.
2. Rinse the 100 ml graduated cylinder three times with 0.01 M KCl standard solutions. Pour 20 ml of the standard into a beaker.

B. Conductivity measurements:

1. Place the conductivity cell in the sample. Slightly agitate the cell to remove any air bubbles. Allow the reading to stabilize. The conductivity reading in units of either mS (milli Siemens) or µS (micro Siemens) will be displayed.
2. Rinse the beaker three times with the test sample. Pour approximately 20 ml of the sample into a 100 ml beaker.
3. Remove the conductivity electrodes from its storage solution and rinse well with reagent water.
4. Immerse the electrodes into the sample solution. Move the electrode up and down three times so that full contact with the sample is made and that any air bubbles are removed.
5. Allow the meter reading to stabilize (approximately 15 seconds) and record the reading.
6. Remove the electrodes, rinse well with the reagent water and place back in its storage solution.
7. Discard the sample and rinse the beaker well with reagent water.

Calculation and reporting:

Report conductivity in µS/cm with a maximum of three significant figures and one decimal place.

Conductivity in µS/cm = (Meter reading in µS/cm) x Dilution factor.

Precautions:

There are no unusual safety hazards associated with this method. Refer to the Quality Manual and SOP of the lab.

Interferences:

Samples containing oil and grease may coat the electrodes producing sluggish readings. Clean the electrodes well between measurements of these samples using iso-propanol followed by distilled water.

References:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017(Method 2510B).
- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 150.1).

(I.4.) Turbidity

(Nephelometry)

Principle:

Turbidity in water is caused by suspended and colloidal matter, such as clay, silt, finely divided organic and inorganic matters, soluble colored organic compounds, plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The more light is scattered the higher is the turbidity. The method is applicable in the turbidity range 0 - 1000 Nephelometric Turbidity Units (NTU).

Equipment:

1. Turbidity meter.
2. Sample tubes.
3. Cleaning tissues.
4. Calibrationsolution 1000, 100, 10, 1.0, 0.02 NTU.

Reagents and material:

1. Turbidity-free water: Pass laboratory reagent grade water through a filter with pore size sufficiently small to remove essentially all particles larger than 0.1 µm.
2. Formazine stock turbidity suspension: Primary ready made standards (1000, 100, 10, 1.0, and 0.02 NTU). Commercially available ready made stock standards (4000 NTU) is used for the preparation of different standards (1000, 100, 50, 10, 1.0 and 0.02 NTU.
3. Laboratory made stock turbidity suspension: It is prepared as follows:
   - Solution (1). Dissolve 1.00 g of hydrazine sulfate in distilled water and dilute to 100 ml.
   - Solution (2). Dissolve 10 g of hexamethylene-tetramine in distilled water and dilute to 100 ml.
4. In a 100 ml volumetric flask, mix 5.0 ml of solution (1) and 5.0 ml of solution (2) and allow standing for 24 h. Dilute to mark and mix. The turbidity of this suspension is 4000 NTU. This suspension is stable for 1 year when properly stored.

5. Standard turbidity suspension: Dilute 25 ml of the stock turbidity suspension to 100 ml with distilled water. This solution has a turbidity of 100 NTU. Dilute 10 times to obtain a standard with a 10 NTU.
6. Two QC check standards (10 and 50 NTU) are used before and after measuring the samples. Refrigerate and store the standards in dark at 4±2 ^{o}C).

Procedure:

Immediately measure turbidity to prevent temperature changes, particle flocculation and sedimentation due to the changing of sample characteristics.

A. Calibration:

1. Rinse the inside of a clean cuvette three times with the sample to be tested.
2. Fill the cuvette with the sample after gentile mixing, and make sure that there is no air bubble.
3. Close the cuvette with the light shield cap. Ensure that all outside surfaces are clean, and dry.
4. Insert the cuvette into the optical well and align it.
5. Record the measured value and store the values.

*B. Turbidity meter calibration***:**

Follow manufacturer's operating instructions for calibration of the used turbidity meter and proceed to sample measurements.

C. Sample analysis:

1. Allow the test solution to acquire the room temperature.
2. Mix the sample solution thoroughly to disperse the solids.
3. Wait until visible air bubbles disappear (a few minutes at most).
4. Pour the sample into a clean dry turbidity vial (if sample has settled, mix gently to re-suspend the solids before pouring into the sample vial). Cap vial securely.
5. Wipe vial free of liquid and fingerprints with a soft lint-free wipe or cloth.
6. Place the calibration solution into the sample chamber and cover with vial cover.
7. Measure the turbidity and similarly repeat with the test solution standard.

Calculation:

$$\text{Sample NTU} = \frac{\text{NTU measured}}{\text{proportion of sample in dilution}}$$

Precautions:

After performing the initial calibration with the primary standard, perform an instrument check by taking measurements of the secondary sealed standard and blank "Turbidity- free water".

1. The performance is good when the blank reads less than the detection limit: (0.03 NTU) and the secondary sealed standard read within ±10 % of the expected values.

2. If results are not within limits, and the blank is higher than the method detection limit (MDL) reanalyze the secondary sealed standard and pass distilled water through membrane filter with small pore size then, reanalyze it.
3. Rapidly settling sand, the presence of air bubbles, and dirty glassware are the main sources of interference and should be removed before analysis.

Interferences:

Rapidly settling sand, the presence of air bubbles and dirty glassware are the main sources of interference and should be removed before analysis.

References:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), 2017 (Method 2130 B).
- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 180.1).

(I.5) Dissolved Oxygen

(*Iodometric Titration-Winkler Method)*

Principle:

Winkler method is the most precise and reliable titrimetric procedure for DO analysis. The method is based on the addition of a divalent manganese solution strong alkali to the water test sample in a glass-stoppered bottle. The DO present, rapidly oxidizes an equivalent amount of the dispersed divalent manganese hydroxide to a higher oxidation state (brownish/orange in color). This brownish-orange color indicates the presence of oxygen. In the presence of iodide and by subsequent acidification, the higher manganese hydroxide revert to the divalent state, while the amount of iodine liberated is equivalent to the original dissolved oxygen content of the sample. The liberated iodine is titrated with a standard solution of sodium thiosulfate using starch as an indicator.

Equipment:

1. Bottles (300 ml) calibrated and equipped with a narrow neck and fitting ground glass stoppers.
2. Pipettes, burettes, and conical flasks.

Reagents:

1. Manganese (Π) sulfate solution:

- Dissolve (480 g) $MnSO_4.4H_2O$, (400 g) $MnSO_4.2H_2O$, or (364 g) $MnSO_4.H_2O$ in distilled water. Filter and dilute to 1 liter.
- The manganese (Π) sulfate solution should not give a color with starch when added to an acidified solution of potassium iodide.

2. 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 1 liter.
- Dissolve 10 g sodium azide (NaN_3) in 40 ml of distilled water). Potassium and sodium salts may be used interchangeably. This reagent should not give color with starch solution when diluted and acidified.

3. Sulfuric acid: One milliliter of concentrated H_2SO_4 is equivalent to about 3 ml of alkali-iodide-azide reagent.
4. Starch indicator: Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid (as a preservative) in 100 ml hot distilled water.
5. Sodium thiosulfate stock solution (0.25 N):

   Dissolve 63 g of sodium thiosulfate pent hydrate: $Na_2S_2O_3.5H_2O$ in 1 liter copper free boiled and cooled distilled water. Add 5 ml chloroform or 2 g NaOH as a preservative.

6. Potassium biiodate (0.0125 N): Dissolve 0.8248 g of potassium biiodate [$KH(IO_3)_2$], previously dried at about 120°C, in distilled water and dilute to 1 liter. The solution is stable for a long period, if stored in a glass-stoppered bottle.
7. Standard sodium thiosulfate titrant (0.025 N):
   - Dilute the stock sodium thiosulfate solution to obtain 0.025 N (100 ml of stock 0.25 N $Na_2S_2O_3$ diluted to 1000 ml with distilled water). Standardize daily.

   - Standardization: Pipette 20 ml of 0.0125 N potassium iodate into a conical flask containing about 100 ml of distilled water. Add 2 ml of concentrated H_2SO_4 followed by about 2 g of solid potassium iodide and immediately titrate against 0.025 N sodium thiosulfate, using starch as indicator.
   - Apply concentration correction in the calculation. When the solutions are of equal strength, 20 ml of 0.025 N $Na_2S_2O_3$ is consumed. If not, adjust the thiosulfate solution to 0.025 N.

Procedure:

1. Fill the DO bottle without turbulently exposing the sample to the air, and put the appropriate stopper on.
2. If the sample contains ferrous iron, add to the sample bottle 0.7 ml of concentrated H_2SO_4, followed by 1 ml of a 0.61% solution of potassium permanganate ($KMnO_4$), and 1 ml of a 40 % solution of potassium fluoride (KF) and mix well by bottle inversion.
3. The amount of permanganate added should be sufficient to obtain a violet tinge that persists for 5 minutes.
4. Remove the excess permanganate by adding 0.5 to 1.0 ml of 2 % potassium oxalate solution ($K_2C_2O_4$).
5. Mix well and let to stand in the dark for 10 minutes to facilitate the reaction. Excess oxalate causes low results; therefore add only an amount of oxalate that completely decolorizes the potassium permanganate in the above mentioned period. After applying this modification, the usual method should be followed.
6. As soon as possible, remove the stopper and add 2 ml manganese sulfate followed by 2 ml alkali-iodide-azide reagent, while dipping the separate pipettes a little below the surface.
7. Stopper carefully to exclude air bubbles and thoroughly mix the contents. To attain this, invert the bottle at least 15 times.
8. Allow the precipitate to settle to the lower one-third of the bottle, repeat the mixing and allow the precipitate to settle completely leaving a clear supernatant liquid.
9. Add 2 ml of concentrated H_2SO_4 immediately after removing the stopper.
10. Stopper, and mix by gentle inversion until all the precipitate dissolves, if it does not, allow standing for a few minutes and repeat the mixing.

11. Pipette 203 ml of the sample for titration (3 ml extra to account for 4 ml or the reagents [manganese(Π) sulfate and alkali-iodide-azide added to the 300 ml of sample] in a conical flask.
12. Immediately titrate the liberated iodine with a standard 0.025 N thiosulfate solution to a pale yellow straw color. Add 2 ml starch solution (giving a blue color) and continue the titration till the first disappearance of the blue color.
13. Disregard subsequent recoloration due to the catalytic effect of nitrite or ferric salts.

Calculation:

For titration of 200 ml sample, 1.0 ml 0.025N $Na_2S_2O_3$ = 1.0 mg DO/l
The results are calculated according to the relation:

DO, (mg/l) = ml of 0.025 N sodium thiosulfate consumed for titration of 203 ml sample.

If the results are desired in milliliters of oxygen per liter of sample at 0 ^oC and 760 mm pressure, multiply mg/l DO by 0.698.

Precautions:

1. It must be remembered that iodine is volatile and therefore the titration must be carried out as fast as possible and with the minimum exposure the air.
2. Don't use synthetic detergents for washing the DO bottles. Normally, the bottles are kept clean by the acidic iodine solution of the Winkler procedure and require no further treatment except thorough rinsing with tap water.
3. New bottles should be cleaned with 5 N sulfuric or hydrochloric acid and then rinsed thoroughly with tap water.
4. Before taking the sample, rinse the sample bottle with a small portion of the sample.
5. The bottle should be completely filled with the sample. Remove bubbles by tapping the neck of the bottle with the stopper.
6. Fix the DO on the site by adding manganese (II) sulfate and alkali-iodide-azide solution, after the permanganate pretreatment, if needed.
7. The titration should be carried out as expediously as possible and with the minimum exposure to air, so as to avoid any losses of iodine.
8. Avoid errors due to careless in collecting the sample, prolonging the completion of the test or due to any interference.

Interferences:

1. Oxidizing or reducing materials that may be present in the sample cause
2. Liberation of iodine from iodide by ozone, chlorine, ferric compounds, and manganese in tri- or higher oxidation states, chromate, persulfate, peroxides. Some reducing agents such as ferrous compounds, thiosulfate, readily oxidizaable organic matter and nitrite reduce iodine.

3. Some organic compounds hinder the settling of the precipitate and some interfere in the color of the starch endpoint. An oxygen meter may be used in these cases.
4. Add 0.7 ml of concentrated H_2SO_4 followed by 1 ml of 0.61 % solution of potassium permanganate and 1 ml of 40 % potassium fluoride solution to avoid the interference of ferrous ion.
5. Remove the excess of potassium permanganate by adding 0.5-1.0 ml of 2 % potassium oxalate solution with well mixing and waiting in the dark for 10 min.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 360.2).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association) APHA), Washington 2017 (Method [4500-O(C)]).

(I.6) Dissolved Oxygen

(Electrometry- Oxygen Electrode)

Principle:

Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with a supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are commonly used because they are permeable to molecular oxygen and are relatively rigid. The "diffusion current" is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by calibration procedures.

Equipment:

Oxygen meter equipped with oxygen sensor (electrode).

Reagents:

1. Sodium thiosulfate stock solution (0.25 N).
2. Sodium thiosulfate solution. Dissolve 18 g of sodium thiosulfate in 300 ml of deionized water and stir until most of the salt has dissolved.

Procedure:

A. Calibration:

1. Air calibration (Auto Cal.):
   - Prepare the calibration sleeve (White Stand) by removing the cap out.
   - Remove the sponge, saturate it with distilled water and squeeze excess water. Replace the sponge into the cap and put the cap onto the stand.
   - Ensure that there are no drops of water on the membrane and follow the manual instruction.

2. Water calibration:

- Water saturated with oxygen is prepared by bubbling (Aeration) of air in stirred deionized water in the beaker for at least one hour.
- Note, the stirrer speed is slow, and then turn off the stirrer.
- Place the probe into the aerated water, calibrate and get the slope. Accepted slope is between 0.6 to 1.2.

3. Probe zero calibration:

- It is recommended to do either air or water calibration before probe zero calibration.

- Prepare an oxygen scavenging solution, such as sodium thiosulfate by adding18 grams of sodium thiosulfate to 300 ml of deionizer water and stirring until most of the salt has dissolved. Place the probe into the sample solution contained in the BOD bottle, place the bottle on the stirrer and stir.
- Allow to eliminate oxygen from membrane for 5 minutes and carry out calibration.
- Gets the slope after D.O reading, and then stabilized D.O will be displayed briefly.

B. Sample measurements:

1. Connect the D.O. probe to the port of the meter.
2. Plug the meter by connection of the cable to the main.
3. Immerse the probe into the sample solution contained in the BOD bottle, and place the bottle on the stirrer and stir.
4. Allow elimination of oxygen from the sensor membrane for 5 minutes and record the reading.
5. After getting a good calibration, put the probe into the test sample and record the reading. Repeat measurements for (2-3) times; get the average of the measure.

Calculation:

D.O. is expressed as mg/l or % saturation.

Precautions:

1. The bottle containing the test sample should be tightly close.
2. The oxygen electrode should be correctly stored.
3. The membrane should be checked from time to time to monitor if there is any rupture.
4. The internal electrolyte solution of the probe should be renewed from time to time.

References:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 4500-OG) membrane electrode method.
- USEPA "Methods for Chemical analysis of Water and Wastes", Ohio, 1983 (Method 360.2).

(I.7) Total Suspended Solids

(Gravimetry)

Principle:

A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C. The filtrate from this method may be used for measuring dissolved solids. The practical range of the determination is 4-20000 mg/l.

Equipment:

1. Glass fiber filters discs, without organic binder.
2. Filter support: Filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
3. Suction flask.
4. Drying oven, 103-105°C.
5. Desiccator.
6. Analytical balance (0.1 mg sensitivity).
7. Weighing dishes or pans.
8. Forceps.

Reagents:

Deionized bidistilled water.

Procedure:

1. Place the glass fiber filter on the membrane filter apparatus or insert into the bottom of a suitable Gooch crucible with wrinkled surface up.
2. While vacuum is applied, wash the disc with three successive 20 ml of distilled water. Remove all traces of water by continuing to apply vacuum.
3. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105°C for one hour.
4. Transfer to a desiccator and store until needed.
5. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter or crucible / filter with forceps or tongs only.
6. For a 4.7 cm diameter filter, filter 100 ml sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm^3 of filter area and collect at least a 1.0 mg of the residue.
7. Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.

8. Shake the sample vigorously and quantitatively transfer the sample volume to the filter using a graduated cylinder. Remove all traces of water by continuing apply vacuum after sample has passed through.
9. With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.
10. Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry for at least one hour at 103 - 105°C. Cool in a desiccator and weigh. Repeat the drying/weighing cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

Calculation:

Total suspended solids, (mg/l) = $\frac{(A-B)}{C} \times 1000$

Where:

A = weight (mg) of filter (or filter and crucible) + residue.

B = weight (mg) of filter (or filter and crucible).

C = volume (ml) of sample filtered.

Precautions:

1. Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
2. Preservation of the sample is not needed, analyse as soon as possible.
3. Refrigeration or icing to 4°C, to minimize micro-biological decomposition of solids, is recommended.
4. Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have affect the results.
5. Samples high in filterable residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter minimizes this potential interference.

References:

- USEPA “Methods for Chemical Analysis of Water and Wastes”, Ohio, 1983 (Method 160.2).
- “Standard Methods for Examination of Water and Wastewater”, 23rd Edition,
- American Public Health Association (APHA), Washington 2017, (Method 2540 D).

(I.8) Total Dissolved Solids

(Gravimetry)

Principle:

A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to a constant weight at 180±2°C. If suspended solids are being determined, the filtrate from the method may be used for residue, filterable. The practical range of the determination is 10 - 20000 mg/l.

Equipment:

1. Glass fiber filter discs, 4.7 cm or 2.1 cm.
2. Filter holder, membrane filter funnel or Gooch crucible adapter.
3. Suction flask, 500 ml.
4. Gooch crucibles, 25 ml (if 2.1 cm filter is used).
5. Evaporating porcelain dishes, 100 ml volume.
6. Water bath.
7. Drying oven, 180±2°C.
8. Desiccator.
9. Analytical balance, 0.1 mg sensitivity.

Reagents:

Deionized bidistilled water.

Procedure:

1. Place the disc on the membrane filter apparatus or insert into the bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water.
2. Remove all traces of water by continuing to apply vacuum after water has passed through and discard washings.
3. Heat the clean dish to 180±2 °C for one hour, cool in a desiccator and store until needed. Weigh immediately before use.
4. Assemble the filtering apparatus and begin suction. Shake vigorously the test sample and rapidly filter 100 ml of the sample. If the total filterable residue is low, a larger volume may be filtered.
5. Filter the sample through the glass fiber filter, rinse with three 10 ml portions of distilled water and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.
6. Transfer 100 ml (or a larger volume) of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.
7. Dry the evaporated sample for at least one hour at 180±2°C. Cool in a

desicccator and weigh. Repeat the drying cycle until a constant weight is obtained or until the weight loss is less than 0.5 mg.

Calculation:

Dissolved solids, (mg/l) = $\frac{(A-B)}{C} \times 1000$

where:

A = weight (mg) of dried residue + dish.

B = weight (mg) of dish.

C = volume (ml) of sample used.

Precautions:

1. Highly mineralized waters and wastewaters containing significant concentrations of calcium, magnesium, chloride and/or sulfate are hygroscopic and require prolonged drying, desiccation and rapid weighing.
2. Samples containing high concentrations of bicarbonate require careful and possibly prolonged drying at 180°C to insure that all the bicarbonate is converted into carbonate.
3. Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.
4. Preservation of the sample is not practical; analysis should begin as soon as possible.
5. Refrigeration or icing to 4°C, to minimize micro-biological decomposition of solids, is recommended.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983(Method 160.1).
- "Standard Methods for Examination of Water and Wastewater", 23rdEdition, American Public Health Association (APHA), Washington 2017, (Method 2540- C).

(I.9) Settleable Solids

(Volumetry)

Principle:

Settleable matter is measured volumetrically with an Imhoff cone. The practical lower limit of the determination is between 0.1 and 1.0 ml/l/h., depending on sample composition.

Equipment:

1. Imhoff cone and stand.
2. Graduated cylinder, class A.
3. Stir-rod made of glass.

Procedure:

1. Fill an Imhoff cone to the 1 liter mark with a well-mixed sample.
2. Allow to settle, gently agitate sample near the sides of the cone with a rod or by spinning.
3. Settle for 15 min or longer as required and record volume of settleable solids in the cone as milliliters per liter of test sample.

Precautions:

1. Do not estimate the floating material as settleable matter.
2. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids, or stir gently and leave to settle.

References:

- USEPA "Methods For Chemical Analysis of Water and Wastes", Ohio, 1983(Method 160.5).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 2540-F).

(I.10) Color

(Visual Colorimetry)

Principle:

Color is determined by visual comparison of the sample color with known concentrations of platinum-cobalt reagent. The unit of color is equivalent to 1 mg platinum/liter in the form of chloroplatinate ion.

Equipment:

1. Nessler tubes, 50 ml.
2. pH-Meter with combined glass-reference electrode.

Reagents:

1. Platinum-cobalt reagent: Dissolve 1.246 g potassium chloroplatinate K_2PtCl_6 (≡ 500 mg Pt) and 1.0 g crystallized cobaltous chloride ($CoCl_2.6H_2O$) in distilled water, add 100 ml concentrated HCl and dilute to 1000 ml with distilled water. This stock standard has a color of 500 units.
2. If K_2PtCl_6 is not available, dissolve 500 mg pure metallic platinum in aqua regea (1+3 concentrated HNO_3 and HCl), remove HNO_3 by repeated evaporation with fresh portions of concentrated HCl. Dissolve this product together with 1 g crystallized $CoCl_2.6H_2O$, and complete as directed above.

Procedure:

1. Prepare standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 70 units by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0 ml stock color standards with distilled water to 50 ml in Nessler tubes.
2. Protect these standards against evaporation and contamination when not in use.
3. Observe sample color by filling a match Nessler tube to the 50 ml mark with sample and comparing it with the standards. Look vertically downwards through tubes towards a white or specular surface placed at such an angle that light is reflected upwards through the columns of liquid.
4. If turbidity was present and has been removed, report as "apparent color". If the color exceeds 70 units, dilute sample with distilled water in known proportions until the color is within the range of the standards.
5. Measure pH of each sample.

Calculation:

1. Calculate color units as follow:

$$\text{Color, (Units)} = \frac{(A \times 50)}{B}$$

where:
A = estimated color of diluted sample.
B = volume (ml) of sample taken for dilution.

2. Report color results in whole numbers and record as follows:

Color units	Record to nearest
1-50	1
51-100	5
101-250	10
251-500	20

3. Report sample pH.

Precautions:

1. The color value of water is highly dependent on pH and invariably increases as the pH of water is raised.
2. Slight turbidity causes the apparent color to be noticeably higher than the true color. Remove turbidity by filtration.
3. Collect samples in clean glassware and perform measurement as soon as possible to avoid variation due to biological activity.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 110.2).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 2120-B).

(I.11) Alkalinity

(Potentiometric Titration)

Principle:

An unaltered sample is potentiometrically titrated to pH 4.5. The sample must not be filtered, diluted, concentrated, or altered in any way. The method is suitable for all ranges of alkalinity.

Equipment:

1. pH-Meter (sensitivity 0.05 pH units). If automatic temperature compensation is not provided, make titration at 25 ± 2°C.
2. Use appropriate size vessel to keep the air space above the solution at a minimum.
3. Use a rubber stopper fitted with holes for the glass electrode, reference electrode (or combination electrode) and burette.
4. Magnetic stirrer, pipettes, flasks and other standard laboratory equipment.
5. Burettes, Pyrex 50, 25 and 10 ml.

Reagents:

1. Sodium carbonate solution, approximately 0.05 N: Place 2.5±0.2 g Na_2CO_3 (dried at 250 °C for 4 hours and cooled in desiccator) into a 1 liter volumetric flask and dilute to the mark.
2. Standard acid (sulfuric or hydrochloric), 0.1 N: Dilute 3.0 ml of concentrated H_2SO_4 or 8.3 ml of concentrated HCl to 1 liter with distilled water.
3. Standardize versus 40.0 ml of 0.05 N Na_2CO_3 solution diluted with about 60 ml distilled water by potentiometric titration to pH of about 5. Lift electrode and rinse into beaker.
4. Boil the solution gently for 3-5 minutes under a watch glass cover. Cool to room temperature.
5. Rinse cover glass into a beaker and continue titration to the pH inflection point.
6. Calculate normality using:

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

where :

A = weight of Na_2CO_3 (g) dissolved in 1 liter.

B = volume of Na_2CO_3 solution used for titration (ml).

C = volume of acid used to reach inflection point (ml).

7. Standard acid (sulfuric or hydrochloric), 0.02 N: Dilute 200.0 ml of 0.1000 N standard acid to 1 liter with distilled water. Standardize by potentiometric titration of 15.0 ml with 0.05 N Na_2CO_3 solution as above.

Procedure:

1. Use a sufficiently large volume of titrant (> 20 ml in a 50 ml burette) to obtain good precision while keeping volume low enough to permit sharp end point.
2. For < 1000 mg $CaCO_3$ / l, use a standard 0.02 N acid titrant. For samples with > 1000 mg $CaCO_3$/l, use a standard 0.1 N acid titrant. A preliminary titration is helpful.
3. For potentiometric titration of normal alkalinity: Place 25 - 50 ml of the sample solution in a conical flask by pipetting with pipette tip near bottom of flask.
4. Measure pH of sample and add standard acid (0.02 or 0.1 N), be careful to stir thoroughly but gently to allow needle to obtain equilibrium.
5. Titrate to pH 4.5. Record volume of titrant.
6. For potentiometric titration of low alkalinity < 20 mg/l, titrate 100-200 ml as above using a 10 ml microburette and 0.02N acid solution.
7. Stop titration at pH in range of 4.3 - 4.7, record titrant volume and exact pH. Carefully add titrant to lower pH by exactly 0.3 pH units and record volume of the titrant.

Calculation:

A. Potentiometric titration to pH 4.5:

$$\text{Alkalinity as } CaCO_3\text{, (mg/l)} = \frac{A \times N \times 50{,}000}{\text{ml of sample}}$$

Where:
A = volume of standard acid consumed in the titration (ml).
N = normality of the standard acid.

B. Potentiometric titration of low alkalinity:

$$\text{Total alkalinity, as } CaCO_3\,\text{(mg/l)} = \frac{(2B - C) \times N \times 50{,}000}{\text{ml of sample}}$$

where:
B = volume (ml) of titrant consumed to first recorded pH.
C = total volume (ml) of titrant used to reach pH 0.3 units lower.
N = normality of acid.

Precautions:

1. The sample should be refrigerated at 4°C and run as soon as practical. Do not open sample bottle before analysis.
2. Substances, such as salts of weak organic and inorganic acids present in large amounts, may cause interference in the electrometric pH measurements.

3. For samples having high concentrations of mineral acids, such as mine wastes and associated receiving waters, titrate to an electrometric endpoint of pH 3.9.
4. Oil and grease, if present coat the pH electrode, and may also interfere by causing sluggish response.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 301.1).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 2320-B).

(II) Inorganic Nonmetallic Constituents

(II.1) Fluoride

(Potentiometry - Fluoride Electrode)

Principle:

Fluoride ion is determined potentiometrically using a fluoride ion selective electrode in conjunction with a standard single junction sleeve-type reference electrode and pH meter having an expanded mill-volt scale or a selective ion meter having a direct concentration scale for fluoride. Concentration levels in the range of 0.1 to 1000 mg/l are measured by this method with minimal interferences from most common ions.

Equipment:

1. Electrometer (pH / mV meter), with expanded scale, or a selective ion meter.
2. Fluoride ion activity electrode.
3. Reference electrode (single junction, sleeve-type) or double junction. Magnetic stirrer.
4. Timer.

Reagents:

1. Total ionic strength adjustment buffer (TISAB), (pH 5.0-5.5): To approximately 500 ml of distilled water in a 1 liter beaker, add 57 ml of glacial acetic acid, 58 g of sodium chloride and 4 g of (1,2-cyclohexylene dinitrilotetraacetic acid) (CDTA). Stir to dissolve, and cool to room temperature. Adjust pH of solution to between 5.0 and 5.5 with 5 N sodium hydroxide (about 150 ml is required). Transfer the solution to a 1 liter volumetric flask and dilute to the mark with distilled water. For work with brines, additional NaCl should be added to raise the chloride level to twice the highest expected level of chloride in the sample. TISAB buffer is commercially available.
2. Stock sodium fluoride solution (1.0 ml ≡ 0.1 mg F): Dissolve 0.2210 g of sodium fluoride in distilled water and dilute to 1 liter in a volumetric flask. Store in chemical-resistant glass or polyethylene bottle.
3. Standard sodium fluoride solution: (1.0 ml ≡ 0.01 mg F): Dilute 100.0 ml of sodium fluoride stock solution to 1000 ml with distilled water.
4. Sodium hydroxide, (5 N): Dissolve 200 g of sodium hydroxide in distilled water, cool and dilute to 1 liter.

Procedure:

1. Prepare a series of standards using the stock fluoride solution in the range of 0 - 2.0 mg/l.
2. Place 50.0 ml of the standard fluoride solution and 50.0 ml of TISAB buffer in a 150 ml beaker Place on a magnetic stirrer and mix at medium speed.
3. Immerse the electrode system (fluoride ion selective electrode and reference electrode) in the solution for at least three minutes or until the reading has stabilized.

4. Record the potential of each standard and plot a calibration graph on a semi logarithmic paper. The concentration of fluoride in mg/l on the logarithmic axis and the potential developed in the solutions on the linear axis.
5. Repeat with 50.0 ml of the test sample, record the potential reading and use the calibration graph for calculation of fluoride ion concentration.

Precautions*:*

1. Extremes of pH interfere; sample pH should be between 4 and 6.
2. Polyvalent cations of Si^{4+}, Fe^{3+} and Al^{3+} interfere by forming complexes with fluoride.
3. The addition of a pH 5.0 buffer containing a strong chelating agent preferentially complexes aluminum (the most common interferent), silicon and iron and eliminates the pH problem.
4. Measurement and solutions storage are preferably carried out in plastic containers.

Interferences*:*

1. Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride and complexing species.
2. CDTA (cyclohexylenediamine tetraacetic acid), a component of the buffer, preferentially complex the interfering cations and release free fluoride ions.
3. Aluminum, the most common interfering species, up to 3.0 mg/l can be complexed preferentially.
4. In acid solutions, F^- forms a poorly ionized acid. The buffer maintains the pH above 5 to minimize hydrogen fluoride complex formation. Hydroxide ion also interferes with the electrode response. The pH maintained by the buffer eliminates hydroxide interference. Distill the sample if the dissolved solids concentration exceeds 10000 mg/l.

References*:*

- USEPA “Methods for Chemical Analysis of Water and Wastes”, Ohio, 1983 (Method 340.2).
- “Standard Methods for Examination of Water and Wastewater”, 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-F (C) Solid).

(II.2) Chloride

(Mercurimetric Titration)

Principle:

An acidified chloride sample is titrated with mercuric nitrate in the presence of mixed diphenylcarbazone-bromophenol blue indicator. The end point of the titration is the formation of the blue-violet mercury diphenylcarbazone complex. The method is suitable for all concentration ranges of chloride content. However, a sample aliquot containing not more than 20 mg chloride is commonly used.

Equipment:

1. Standard laboratory titrimetric equipment including a 1.0 ml or 5.0 ml microburette with 0.01 ml graduations.
2. Standard laboratory glassware.
3. A microburette 10 ml (0.01 ml divisions).
4. Magnetic stirrer and stirring bar.
5. Erlenmeyer flask, 250-ml.

Reagents:

1. Standard sodium chloride, (0.025 N): Dissolve 1.4613±0.02 g (dried at 600°C for 1 hour) in chloride-free water in a 1 liter volumetric flask and dilute to the mark (1.0 ml ≡ 886.5 μg Cl).
2. Nitric acid, HNO_3 solution (3+997).
3. Sodium hydroxide solution, NaOH, (10 g/l). Potassium iodide at pH 4 or less. The iodine is titrated with a standard reducing agent such as sodium thiosulfate or phenyl arsine oxide using a starch indicator. The results are calculated as mg/l Cl even though, the actual measurement is of total oxidizing power because chlorine is the dominant oxidizing agent present.
4. Hydroquinone solution (10 g/l): Dissolve 1 g of purified hydroquinone in water in a 100 ml volumetric flask and dilute to the mark.
5. Mercuric nitrate titrant (0.141 N): Dissolve 25 g $Hg(NO_3)_2.H_2O$ in 900 ml of distilled water acidified with 5.0 ml concentrated HNO_3 in a 1 liter volumetric flask and dilute to the mark with distilled water. Filter if necessary. Standardize against standard sodium chloride solution. Adjust to exactly 0.141 N and check. Store in a dark bottle, A 1.00 ml aliquot of this solution equivalent to 5.00 mg of chloride.
6. Mercuric nitrate titrant (0.025 N) and (0.0141 N) are prepared by dilution of the stock solution.
7. Mixed indicator reagent: Dissolve 0.5 g crystalline diphenylcarbazone and 0.05g bromophenol blue powder in 75 ml 95% ethanol in a 100 ml volumetric flask and dilute to the mark with 95 % ethanol. Store in brown bottle and discard after 6 months.

Procedure:

1. Use 50 ml of sample or an aliquot of sample diluted to 50 ml with distilled water, so that the concentration of chloride does not exceed 20 mg/aliquot.
2. Use 0.025 N mercuric nitrate titrant, if the sample or aliquot contains more than 2.5 mg of chloride and 0.0141 N mercuric nitrate titrant if the sample or aliquot contains less than 2.5 mg of chloride.
3. Determine an indicator blank on 50 ml chloride -free water.
4. Concentrate an appropriate volume to 50 ml, if the sample contains less than 0.1 mg/l chloride.
5. Add 5 drops of mixed indicator reagent shake or swirl solution.
6. If a blue-violet or red color appears add HNO_3 solution dropwise until the color changes to yellow.
7. If a yellow or orange color forms immediately on addition of the mixed indicator, add NaOH solution dropwise until the color changes to blue-violet then add HNO_3 solution dropwise until the color changes to yellow.
8. Add 1 ml excess of HNO_3 solution.
9. Titrate with standard mercuric nitrate titrant until a blue-violet color persists throughout the solution.

Calculation:

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

where:

A = volume (ml) of mercuric nitrate titrant for sample.

B = volume (ml) of mercuric nitrate titrant for the blank.

N = normality of mercuric nitrate titrant.

[NaCl, (mg/l) = chloride, (mg/l) x 1.65]

Precautions:

1. Sulfite interference can be eliminated by oxidizing a 50 ml aliquot of sample solution with 0.5 to 1 ml of H_2O_2.
2. If chromate is present and iron is not present, the end point may be difficult to detect an olive-purple color.
3. If chromate is present at > 100 mg/l and iron is not present, add 2 ml of fresh hydroquinone solution.
4. If ferric ion is present, use sample volume containing no more than 2.5 mg of ferric ion or ferric ion plus chromate ion. Add 2 ml of fresh hydroquinone solution.

Interferences:

Bromide and iodide are titrated with $Hg(NO_3)_2$ in the same manner as chloride.

Chromate, ferric and sulfate ions interfere when present at levels above 10 mg.

References:

- USEPA "Methods For Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 325.3).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-Cl^- (C).

(II.3) Chloride

(Argentimetric Titration)

Principle:

In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before the red silver chromate is formed.

Equipment:

1. Erlenmeyer flask, 250-ml.
2. Burette, 50-ml.

Reagents:

1. 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 liter with distilled water.
2. Standard silver nitrate titrant, 0.0141 M (0.0141 N): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 ml. Standardize against NaCl by the procedure described below; 1.00 ml ≡ 500 μg Cl^-. Store in a brown bottle.
3. Standard sodium chloride, 0.0141 M (0.0141 N): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 ml; 1.00 ml ≡ 500 μg Cl^-.
4. Aluminum hydroxide suspension reagent for removal of interferences: Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 12H_2O$, in 1 liter distilled water. Warm to 60°C and add 55 ml of concentrated aqueous ammonia (NH_4OH) slowly with stirring. Let stand for about 1 h, transfer to a large bottle, and wash the precipitate by successive additions, with thorough mixing and decanting, of distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 liter.
5. Phenolphthalein indicator solution.
6. Sodium hydroxide, NaOH, 1 N.
7. Sulfuric acid, H_2SO_4, 1 N.
8. Hydrogen peroxide, H_2O_2, 30 %.

Procedure:

1. Sample preparation: Use a 100-ml sample aliquot or a suitable portion diluted to 100 ml. If the sample is highly colored, add 3 ml $Al(OH)_3$ suspension, mix, let to settle, and filter. If sulfide, sulfite, or thiosulfate is present, add 1 ml H_2O_2 and stir for 1 min.
2. Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode.

3. Determine the amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.
4. Add 1.0 ml of K_2CrO_4 indicator solution and titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition.
5. Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 ml is usual.

Calculation:

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

where:
A = ml titration for sample.
B = ml titration for blank. and
N = normality of Ag NO_3.
(mg NaCl/l = (mg Cl^-/l) × 1.65.

Interference:

1. Substances in amounts normally found in potable waters will not interfere.
2. Bromide, iodide, and cyanide register as equivalent chloride concentrations.
3. Sulfide, thiosulfate, and sulfite ions interfere but can be removed by treatment with hydrogen peroxide.
4. Orthophosphate in excess of 25 mg/l interfere by precipitating as silver phosphate. Iron in excess of 10 mg/l interferes by masking the end point.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-Cl^- B].

(II.4) Chlorine, Total Residual

(Spectrophotometry - DPD Method)

Principle:

Chlorine (hypochlorite ion, hypochlorous acid) and chloramines stoichiometrically liberate iodine from potassium iodide at pH 4 or less. The liberated iodine reacts with N, N-diethyl-*p*-phenylenediamine (DPD) to produce a red colored solution with a maximum absorbance at 515 nm. The method is applicable to the concentration range of 0.2 - 4 mg/l chlorine.

Equipment:

Spectrophotometer for use at 515 nm and cells of light path 1.0 cm or longer.

Reagents:

1. Phosphate buffer solution: Dissolve 24 g anhydrous disodium hydrogen phosphate, Na_2HPO_4, and 46 g anhydrous potassium dihydrogen phosphate, KH_2PO_4, in distilled water. Dissolve 800 mg disodium ethylenediamine tetraacetate dihydrate in 100 ml distilled water. Combine these two solutions and dilute to 1 liter with distilled water. Add 20 mg $HgCl_2$ as a preservative.
2. N,N-Diethyl-*p*-phenylenediamine (DPD) indicator solution : Dissolve 1 g DPD oxalate or 1.5 g *p*-amino-N,N-diethylaniline sulfate in chlorine free distilled water containing 8 ml of (1+3) H_2SO_4 and 200 mg disodium ethylenediamine tetraacetate dihydrate. Dilute to 1 liter, store in a colored, glass stoppered bottle. Discard when discolored. The buffer and indicator sulfate are available as a combined reagent in stable powder form.
3. Sulfuric acid solution (1+3): Slowly add one part of H_2SO_4 (sp.gr. 1.84) to three parts of distilled water.
4. Potassium iodide, KI crystals.
5. Stock potassium permanganate solution: Place 0.891 g of $KMnO_4$ in a volumetric flask and dilute to 1 liter.
6. Standard potassium permanganate solution: Dilute 10.00 ml of stock potassium permanganate solution to 100 ml with distilled water in a volumetric flask. One milliliter of this solution diluted to 100 ml with distilled water is equivalent to 1.00 mg/l chlorine.

Procedure:

A. Calibration:

1. Prepare a series of permanganate standards covering the chlorine equivalent range of 0.05 to 4 mg/l.
2. Place 5 ml phosphate buffer in a flask.

3. Add 5 ml DPD reagent.
4. Add 100 ml permanganate standard.
5. Read at 515 nm on a spectrophotometer and record the absorbance.
6. Return the contents of the cell to the flask.
7. Titrate the contents of the flask with standard ferrous ammonium sulfate until the red color is discharged. Record the results.

B. Sample analysis:

1. Place 0.5 ml phosphate buffer in a flask.
2. Add 0.5 ml DPD reagent.
3. Add approximately 0.1 g KI.
4. Add 10 ml of sample.
5. Let stand for 2 minutes.
6. Read at 515 nm, and record the absorbance.

Calculation:

1. Plot the absorbance of the standard permanganate solutions on the vertical axis versus the titrated concentration on the horizontal axis.
2. Draw the line of best fit through the points.
3. Locate the absorbance of the sample on the vertical axis.
4. Read the concentration on the horizontal axis at the intersect of the absorbance and the calibration line.

Precautions:

1. Interferences are caused by any oxidizing agent but these are usually present at insignificant concentrations compared to the residual chlorine concentrations.
2. Turbidity and color will essentially prevent the colorimetric analysis.

Interferences:

The method is subject to interference by oxidized forms of manganese unless compensate for by a blank. Nitrogen dichloride, if present may react partially as free chlorine. Interference from high concentration of monochloramine which is avoided by adding thioacetamide immediately after reagent addition. Compensate for color and turbidity by using sample to zero photometer.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 330.5).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-Cl^- (G)].

(II.5) Ammonia-Nitrogen

(Potentiometry - Ammonia Gas Electrode)

Principle:

Ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution. Dissolved ammonia is converted to ammonia gas by raising the pH to above 11 with a strong base. Diffusion of ammonia through the membrane of the electrode causes change of the pH of the internal solution that is sensed by the pH electrode inside the ammonia sensor. Sample distillation is unnecessary. The method covers the range from 0.03 to 1400 mg NH_3/l in potable, surface, domestic and industrial wastes.

Equipment:

1. Electrometer: A pH meter with expanded millivolt scale capable of 0.1 mV resolution between - 700 mV and + 700 mV or a specific ion meter.
2. Ammonia-selective electrode.
3. Magnetic stirrer, thermally insulated, with Teflon-coated stirring bar.

Reagents:

1. Ammonia free water.
2. Sodium hydroxide, 10 N.
3. NaOH/EDTA solution, 10 N: Dissolve 400 g NaOH in 800 ml water. Add 45.2 ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate ($Na_4EDTA.4H_2O$) and stir to dissolve. Cool and dilute to 1000 ml.
4. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl (dried at 100°C) in water, and dilute to 1000 ml; 1.00 ml ≡ 1.00 mg N ≡ 1.22 mg NH_3.
5. Standard ammonium chloride solutions.

Procedure:

1. Preparation of standards: Prepare a series of standard ammonium solutions covering the concentration of 1000, 100, 10, 1.0, and 0.1 mg NH_3-N/l by making decimal dilutions of stock NH_4Cl solution with water.
2. Electrode calibration: Place 100 ml of each standard solution in a 150 ml beaker. Immerse an ammonia electrode in a standard of lowest concentration and mix using a magnetic stirrer. Limit stirring speed to minimize possible loss of ammonia from the solution. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add a sufficient volume of 10 N NaOH solution (1 ml is usually sufficient) to raise pH above 11. If silver or mercury is present, use NaOH/EDTA solution in place of NaOH solution. If it is necessary to add more than 1 ml of either NaOH or NaOH/ EDTA solution, record the volume and use it in subsequent calculations. Keep the electrode in solution until stable millivolt reading is

obtained. Do not add NaOH solution before immersing electrode, because ammonia may be lost from a basic solution. Repeat procedures with the remaining standards, proceeding from lowest to highest concentration. Wait until the reading has stabilized (at least 2 to 3 minutes) before recording millivolts for standards and samples containing less than 1 mg NH_3-N/l.

3. Preparation of standard curve: Using semi-logarithmic graph paper, plot ammonia concentration in milligrams [NH_3-N per liter] on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly a tenfold change of NH_3-N concentration will produce a potential change of about 59 mV.
4. Calibration of specific ion meter: Refer to manufacturer's instructions.
5. Measurement of samples: Dilute the test sample, if necessary, to bring NH_3-N concentration to within calibration curve range. Place 100 ml of the test sample solution in 150 ml beaker and follow up the calibration procedure mentioned above. Record volume of 10 N NaOH added. Read NH_3-N concentration from standard curve.

Calculation*:*

$$\text{NH}_3\text{-N, (mg/l)} = A \times B \times \frac{100 + D}{100 + \text{C}}$$

where:

A = dilution factor.

B = concentration of NH_3-N (mg/l) from calibration curve.

C = volume (ml) of 10 N NaOH added to calibration standards.

D = volume (ml) of 10 N NaOH added to sample.

Interferences:

1. Amines cause positive interference.
2. Mercury and silver interfere by complexing with ammonia, unless the NaOH /EDTA solution is used.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 350.3).
- "Standard Methods for Examination of Water and Wastewater", 23rdEdition, American Public Health Association (APHA), Washington 2017 (Method 4500-NH_3 (D).

(II.6) Ammonia - Nitrogen

(Visual Titrimetry - Kjeldahl Method)

Principle:

Kjeldahl method is the standard method of nitrogen determination. The method consists of two basic steps: (1) Distillation of ammonia into a trapping solution; (2) Quantification of ammonia by titration with a standard solution. The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent. Ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg NH_3-N/l. The distillation and titration procedure are used especially for NH_3-N concentrations greater than 5 mg/l. Use boric acid as the absorbent following distillation if the distillate is to be titrated.

Sample collection, preservation and handling:

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h of collection, refrigerate unacidified sample at 4 °C. For preservation for up to 28 d, freeze at -20 °C unacidified, or preserve samples by acidifying to pH < 2 and store at 4 °C. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination.

CAUTION: Although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

Reagents:

1. Distilled water.
2. NaOH solution, (35 %).
3. H_2SO_4, (0.01 M) titrant.
4. NaOH solution, (1 M) as neutralizing and dechlorinating agent.
5. Boric acid solution, (4 %).
6. Dissolve 40 g of boric acid (H_3BO_3) in deionized water and dilute to 1 liter, add 10 ml mixed indicator solution (purple color), prepare monthly.
7. Mixed indicator: Mix 2 volumes of 0.2 % methyl red in 95 % ethyl alcohol with one volume of 0.2 % methylene blue in 95% ethyl alcohol. This solution should be prepared fresh every 30 days.

Equipment:

1. Distillation apparatus by Gerhardt vapodest 20 s).
2. Glass 250 to 300 ml Kjeldahl flasks.
3. Pipette.
4. Graduated cylinder.

5. Stirring rode (magnet).
6. Stirrer.
7. Erlenmeyer flask.
8. Burette 25 ml.

Procedures:

1. Setup of the of equipment: Add 200 ml of distilled water to a 300 ml Kjeldahl tube. Add some boiling chips which have been previously treated with dilute NaOH to prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia with the reagent.
2. Sample preparation: Neutralize and remove the residual chlorine in the sample by adding dechlorinating agent equivalent to the residual chlorine.
3. To 250 ml of the test sample, add 1M NaOH until the pH becomes 12.5, checking the pH during addition with a pH meter.
4. Select a suitable sample volume as described in Table (II.1).

Table (II.1) The volume of test sample used accoding to their ammonia concentrations

Ammonia-nitrogen in the sample, (mg/l)	Sample volume, (ml)
5 – 10	250
10 – 20	100
20 – 50	50
50 – 100	25

* If NH_3 is more than 100 mg/l, dilute or use lesser volume than 25 ml. But do not use less than 5 ml sample solution.

1. Turn on the Gerhardt distillation apparatus and cooling water.
2. Add 25 ml boric acid to an Erlenmeyer flask.
3. Add selected volume of test sample into glass test tubes.
4. Place one hose in 35 % NaOH solution and the other hose in distilled water.
5. Distill for 4 minutes or until the color of boric acid turns purple to green.
6. Titrate with 0.01 M H_2SO_4 until the color reach, the original purple color of boric acid at the beginning of titration.

Calibration:

1. Prepare stock 1000 mg-N (as NH_3/l) calibration standard solution: In a 1 liter volumetric flask, dissolve 3.819 g of ammonium chloride (NH_4Cl), previously dried for one hour at 105°C, in about 500 ml deionized water. Add 1 ml concentrated H_2SO_4 and dilute to the mark.

2. Intermediate 100 mg-N (as NH_3/l) calibration standard: In a 1 liter volumetric flask, dilute 100.0 ml of the stock calibration standard (a) in about 500 ml water. Add 1 ml H_2SO_4 and dilute to the mark with deionized water.

   This solution is used also as spike solution.

   - Working calibration standards: Prepare standards over the range of analysis. For the working range of 0 - 2.00 mg-N (as NH_3), the following standards may be used.
   - Stock 100 mg/l ammonia control standard: (Any ammonia compound may be used for the control standards). In a one liter volumetric flask, dissolve 0.4717 g of ammonia sulfate [$(NH_4)_2SO_4$], previously dried at 105 °C for one hour, in about 500 ml deionized water. Add 1 ml of concentrated H_2SO_4 and dilute to the mark.
   - Working control standards (Table II.2) are typical:

Table (II.2) Working control standards

Volume (ml) of stock control standard diluted to 1 liter	Concentration, mg/l
CS-1 2.0	0.20
CS-2 6.0	0.06

Calculation:

$$NH_3\text{– N /l, mg} = \frac{(A-B)\times 280}{\text{ml of sample}}$$

where:

A = volume of H_2SO_4 titrated for sample, ml.

B = volume of H_2SO_4 titrated for blank, ml.

Precautions:

1. Handle acid safely: use acid resistant fume hood. Always add acid to water unless otherwise directed in the method. Wear face shield and heavy gloves to protect against splashes. If acids are spilled on skin, immediately wash with large amounts of water.
2. Sulfuric acid and sodium hydroxide can burn skin, eyes and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated acid or alkali. Use effective fume removal device to protect against acid fumes or alkali dusts or vapors. Always add concentrated sulfuric acid or sodium hydroxide pellets to water, not vice versa. Concentrated sodium hydroxide can quickly and easily cause blindness. If splashed on skin or in eyes, flush with copious amounts of water and seek medical attention. Keep baking soda and vinegar handy in case of chemical spills.
3. Sulfur oxide fumes produced during digestion are hazardous to breathe. Do not inhale.
4. Sample digestate must be cooled down before dilution with water to avoid a violent reaction and acid shooting out of the flask. Likewise, the diluted digest must be cooled before addition of sodium hydroxide to avoid a similarly violent reaction.
5. Method detection limit (MDL): NH_3– N/l: 0.062 mg/l

Interferences*:*

1. Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly to give ammonia in the solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5.
2. The hydrolysis amounts to about 7 % at this pH for urea and for about 5% for cyanates. Volatile alkaline compounds such as hydrazine and amines will influence the titrimetric results. Residual chlorine reacts with ammonia and should be removed by sample pretreatment. If the sample is likely to contain residual chlorine, treat immediately upon collection with dechlorinating agent.

References:

- American Association of Cereal Chemists. Approved Methods of Analysis. St. Paul, MN: American Association of Cereal Chemists, 1995.
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), 2017.
- AOAC International (formerly the Association of Official Analytical Chemists). Official Methods of Analysis. Arlington, VA: AOAC International, 1995.
- Bradstreet, R. B. The Kjeldahl Method for Organic Nitrogen. New York, NY: Academic Press Incorporated, 1965.
- Jones, J. Benton. Kjeldahl Method for Nitrogen Determination. Athens, GA: Micro-Macro Publishing, 1991.
- Kjeldahl, Johan Z. A new method for the determination of nitrogen in organic bodies. Analytical Chemistry 22 (1883): 366.
- Meloan, Clifton E., and Pomeranz, Y. Food Analysis: Theory & Practice. Westport, CT: AVI Publishing Company, 1978.
- United States Environmental Protection Agency. Methods for Chemical Analysis of Water and Wastes. Cincinnati, OH: Environmental Protection Agency, EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, 1979.

(II.7) Ammonia-Nitrogen

(Spectrophotometry - Phenate Method)

Principle:

An intensely blue compound of indophenols is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

Equipment:

Spectrophotometer, for use at 640 nm with a light path of 1.0 cm or greater.

Reagents:

1. Phenol solution: Mix 11.1 ml of liquefied phenol (≥ 89%) with 95 % v/v ethyl alcohol to a final volume of 100 ml, the solution is weekly prepared. (Caution: Wear gloves and eye protection when handling phenol; use good ventilation area to minimize all personnel exposure to this toxic volatile substance.
2. Sodium nitroprusside, 0.5 % w/v: Dissolve 0.5 g sodium nitroprusside crysttals in 100 ml deionized water. Store in an amber bottle for up to 1 month.
3. Alkaline citrate: Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized water. Dilute to 1000 ml.
4. Sodium hypochlorite, commercial solution, about 5%. This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.
5. Oxidizing solution: Mix 100 ml alkaline citrate solution with 25 ml sodium hypochlorite. Prepare fresh daily.
6. Stock ammonium solution.
7. Standard ammonium solution: Use stock ammonium solution and water to prepare a calibration curve in a range appropriate for the concentrations of the samples.

Procedure:

1. To a 25-ml sample in a 50-ml Erlenmeyer flask, add, with thorough mixing after each addition, 1.0 ml phenol solution, 1.0 ml sodium nitroprusside solution, and 2.5 ml oxidizing solution.
2. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature (22 to 27°C) in subdued light for at least 1 h. The color is stable for 24 h.
3. Measure absorbance at 640 nm. Prepare a blank and at least two other standards by diluting the stock ammonia solution into the sample concentration range. Similarly treat the standards and test samples.

Calculation:

Prepare a standard curve by plotting absorbance readings of standards against ammonia concentrations of the standards. Compute sample concentration by comparing sample absorbance with the standard curve.

Interferences:

Complexing magnesium and calcium with citrate eliminates interference produced by precipitation of these ions at high pH. There is no interference from other trivalent forms of nitrogen. Remove interfering turbidity by distillation or filtration. If hydrogen sulfide is present, remove by acidifying samples to pH 3 with dilute HCl and aerating vigorously until sulfide odor no longer can be detected.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-NH_3 (F)].

(II.8) Nitrite-Nitrogen (NO_2-N)

(***Spectrophotometry - Griess Reaction Method)***

Principle:

Nitrite reacts in strongly acid medium with sulfanilamide. The resulting diazo compound is coupled with N-(1-naphthyl)-ethylenediamine-dihydrochloride to form an intensely red-coloured azo-compound. The absorption of the dye is proportional to the concentration of nitrite present. The method is applicable in the range of 0.01 to 1.0 mg nitrite-nitrogen/l. Samples containing higher concentration must be diluted before analysis. The method is suitable for determining the concentration of 5 to 1000 µg NO_2/liter.

Equipment*:*

1. Spectrophotometer.
2. Nessler tubes (50 ml) and 1.0 cm glass cuvettes.

Reagents*:*

1. Color reagent: To 800 ml water, add 100 ml 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl) - ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 lilter with water. The solution is stable for about one month when stored in a dark bottle in refrigerator.
2. Preparation of the stock solution: Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 ml (1.00 ml ≡ 210 µg N). Preserve with 1 ml $CHCl_3$.
3. Working standard solution: Dilute 10.0 ml of the stock solution to 1 liter of distilled water (1.0 ml ≡ 2.1 µg nitrite-N).

Procedure*:*

1. Filter the test sample, if necessary, through a 0.45 µm pore-size membrane filter.
2. Put 10-50 ml of the sample in a 50 ml Nessler tube.
3. Prepare a series of standards in 50 ml Nessler tubes (1.0 - 20.0 µg of nitrite-N per tube).
4. Add 2 ml of buffer color reagent to each standard and sample, mix and allow color to develop for at least 15 min.
5. Check the pH of the solution to ensure that the value is between pH 1.5 and 2.0.
6. Measure the absorbance of the standard and sample in the spectrophotometer at 540 nm against a blank.
7. Plot the amount of nitrite-nitrogen per standard tube against the absorbance.
8. Read the concentration of nitrite-nitrogen per sample tube from the calibration curve.

Calculation:

$$\text{Nitrite-nitrogen, } (\mu g/l) = \frac{1000 \times \text{reading graph } (\mu g \text{ nitrite - nitrogen})}{\text{volume of sample (ml)}}$$

Precautions*:*

1. The nitrite concentration in collected samples can change very rapidly due to bacterial oxidative or reductive conversions and the analysis should be carried out as early as possible.
2. Short time preservation for one or two days is possible by deep freezing (-20°C) or the addition of 40 mg mercuric ion/ liter of sample and storing at 4°C.

Interferences:

1. Chemical incompatibility makes it unlikely that NO_2, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added.
2. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, chloroplatinate ($PtCl_6^{2-}$), and metavanadate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt.
3. Colored ions should be absent. Remove suspended solids by filtration.

References*:*

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 354.1).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-NO_2 (B).

(II.9) (Nitrate and Nitrite)-Nitrogen

(Spectrophotometry - John Reduction Method)

Principle:

Nitrate is reduced to nitrite upon passing through a column containing copper amalgamated cadmium granules or filings. Nitrite if originally present, plus reduced nitrate) are determined by the same spectrophotometric method (Griess reaction) as described earlier for nitrite. The applicable range of this method is 0.01 to 1.0 mg/l nitrate-nitrite nitrogen. The range of application is 0.01 to 1.0 mg for NO_3 levels. The range can be extended by sample dilution.

Equipment:

1. Reduction columns: Constructed from three pieces of glass tubing joined end to end.
2. All glass ware needed for nitrite analysis.
3. Spectrophotometer with 1.0 cm glass cuvettes.

Reagents:

1. Prepare copper - cadmium granules by washing 25 g new or used 40 - 60 mesh cadmium metal (Cd) granules with 6 N HCl and rinse with water. Swirl the cadmium granules with 100 ml 2 % $CuSO_4$ solution for 5 minutes or until the blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with water to remove all precipitated copper metal (Cu).
2. Buffer - color reagent: Put 105 ml of hydrochloric acid in a glass stoppered dark bottle. Add 5.0 g sulfanilamide and 0.5 g of N- (1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved. Add 136 g of sodium acetate and again stir until dissolved. Dilute to 500 ml with distilled water. This solution is stable for several weeks if stored in the dark (wrap in aluminum foil).
3. Ammonium chloride - EDTA solution: Dissolve 13 g of NH_4Cl and 1.7 g of (EDTA) in 900 ml distilled water. Adjust the pH to 8.5 with concentrated aqueous ammonia and dilute to one liter.
4. Diluted ammonium chloride-EDTA solution: Dilute 300 ml NH_4Cl-EDTA solution to 500 ml with water.
5. Hydrochloric acid: HCl, 6 N.
6. Copper sulfate solution, 2 %.
7. Stock nitrite solution: Dissolve 0.4926 g of previously dried anhydrous sodium nitrite (24 hours in a desiccator) in distilled water and dilute to one liter. Preserve with 2 ml chloroform per litre (1.0 ml ≡ 100 μg nitrite-N).

8. Test nitrite solution: Working standard solution is prepared by diluting the stock solution to 1 litre with distilled water (1.0 ml ≡ 1.0 μg nitrite-N).
9. Stock nitrate solution: Dissolve 7.218 g of KNO_3 in distilled water, and dilute to 1 litre. Addition of 2 ml of chloroform allows the storage of the solution for up to 6 months (1.0 ml ≡ 1.0 mg NO_3-N).
10. Standard nitrate solution: Dilute 10 ml of the stock nitrate solution to 1 liter (1.0 ml ≡ 10 μg NO_3-N).

Procedure:

1. Preparation of reduction column: Plug the bottom of the reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column of 18.5 cm long. Maintain the water level above Cu-Cd granules to prevent entrapment of air. Wash the column with 200 ml of diluted NH_4Cl-EDTA solution. Activate the column by passing through it, at least 100 ml of a solution consisting of 25 % 1.0 mg NO_3-N/l, at a rate of 7 to 10 ml/min.
2. Filter the sample through a 0.45 μm pore size membrane filter.
3. Adjust the pH to between 7 and 9, using NaOH or HCl as necessary. This ensures a pH of 8.5 after adding NH_4Cl-EDTA solution.
4. To 25 ml sample or a portion diluted to 25 ml, add 75 ml NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 ml/min, discard the first 25 ml and collect the rest in the original sample flask. There is no need to wash columns between samples, but if the columns are not to be used for several hours or longer, pour 50 ml dilute NH_4Cl-EDTA solution on, and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.
5. Carry out the reduction of NO_3-N standards exactly as prescribed for the samples. At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration to verify the efficiency of the reduction column.
6. As soon as possible, and not more than 15 min after reduction, add 2.0 ml color reagent to 50 ml of the sample or standard and mix. Between 10 min and 2 hours afterward, measure absorbance at 543 nm against a distilled water reagent blank. Note: if the (NO_3+NO_2)-N concentration exceeds the standard curve range (about 1 mg N/l), use the remainder of reduced sample to make an appropriate dilution and analyze again.
7. Make a calibration curve relating the absorbance of the nitrate standards against the amount of (NO_3+NO_2) present. Read the amount of (NO_3+NO_2) in a sample from the graph.

Calculation:

$$(NO_3+NO_2)\text{ - N, (mg/l)} = \frac{\text{reading graph } (\mu g)}{\text{volume of sample (ml)}}$$

Precautions:

1. To prevent any change in the nitrate it should be determined immediately after sampling. If storage is necessary, the sample should be kept at 4 ^{o}C with or without preservatives such as H_2SO_4 (0.8 ml of concentrated H_2SO_4/l of sample).
2. Buildup of suspended matter in the reduction column will restrict sample flow. Since nitrate nitrogen occurs in a soluble state, the sample can be prefiltered.
3. Highly turbid samples should be treated with zinc sulfate before filtration to remove turbidity.
4. Low results might be obtained for samples that contain high concentrations of iron, copper, cadmium or other metals. EDTA is added to eliminate this interference.

Interferences*:*

Sample turbidity may interfere. Remove by filtration before analysis. Sample color that absorbs in the photometric measuring range used for analysis interfere.

References*:*

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 353.3).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-NO_3 (E)].

(II.10) Nitrate-Nitrogen

(Spectrophotometry - Direct Ultraviolet)

Principle*:*

This technique is only used for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO_3 calibration curve follows Beer's law up to 11 mg N/l. UV absorption measurement at 220 nm enables rapid determination of NO_3. Since dissolved organic matter may also absorb at 220 nm. Since NO_3 does not absorb at 275 nm, a second measurement due to organic compounds is made at 275 nm and used to correct the NO_3 value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended, if a significant correction from organic matter absorbance is required, although it may be useful in monitoring nitrate levels within a water body with a constant type of organic matter. Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original nitrate content by another method. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1 N HCl is designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg $CaCO_3$/l. Chloride has no effect on the determination. This method is applicable for uncontaminated natural waters and potable water supplies

Equipment:

Spectrophotometer, for use at 220 nm and 275 nm with matched silica cells of 1.0-cm or longer light path.

Reagents:

1. Nitrate-free water*:* Use redistilled, distilled or deionized water of highest purity to prepare all solutions and dilutions.
2. Stock nitrate solution*:* Dry potassium nitrate (KNO_3) in an oven at 105 ºC for 24 h. Dissolved 0.7218 g in water and dilute to 1000 ml; 1.00 ml ≡ 100 µg NO_3-N. Preserve with 2 ml $CHCl_3$/l. This solution is stable for at least 6 months.
3. Intermediate nitrate solution*:* Dilute 100 ml stock nitrate solution to 1000 ml with water (1.00 ml ≡ 10.0 mg NO_3-N). Preserve with 2 ml $CHCl_3$/l. This solution is stable for 6 months.
4. Hydrochloric acid solution, HCl, 1 N: Dilute 83 ml of concentrated HCl to 1 liter with water, store in glass or high-density polyethylene (HDPE) bottle.
   The solution is stable for 1 year if kept in a closed bottle.

Procedure:

1. Treatment of sample: To 50 ml clear sample, filtered if necessary, add 1 ml HCl solution and mix thoroughly.
2. Preparation of standard curve: Prepare NO_3 calibration standards in the range 0 to 7 mg NO_3-N/l by diluting the following volumes of intermediate nitrate solution: 0.00, 1.00, 2.00, 4.00, 7.00 ….. 35.0 ml to 50 ml.
3. Spectrophotometric measurements: Read absorbance or transmittance against redistilled water set at zero absorbance or 100 % transmittance. Use a wavelength of 220 nm to obtain NO_3 reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

Calculation:

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3. Construct a standard curve by plotting absorbance due to NO_3 against NO_3-N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. Note: If correction value is more than 10 % of the reading at 220 nm, do not use this method.

Interferences:

Dissolved organic matter, surfactants, NO_2, and Cr^{6+} ions interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate, may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation of individual correction curves.

References:

- Standard Methods for Examination of Water and Wastewater‖, 23 rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-NO3 (B).
- Hoather, R.C. & R.F. Rackman. 1959. Oxidized nitrogen and sewage effluents observed by ultraviolet spectrophotometry. Analyst 84:549.
- Goldman, E. & R. Jacobs. 1961. Determination of nitrates by ultraviolet absorption. J. Amer. Water Works Assoc. 53:187.
- Armstrong, F.A.J. 1963. Determination of nitrate in water by ultraviolet spectrophotometry. Anal. Chem. 35:1292.
- Navone, R. 1964. Proposed method for nitrate in potable waters.J. Amer. Water Works Assoc. 56:781.

(II.11) Sulfate

(Turbidimetry)

Principle:

Sulfate ion is determined by convertion into barium sulfate under controlled conditions and measured turbidometry. The resulting turbidity is determined by a nephelometer, filter photometer or spectrophotometer and compared with a calibration curve prepared from standard sulfate solutions. The method is suitable for all concentration ranges of sulfate. However, reliable results are obtained for solutions containing not more than 40 mg SO_4/l. The minimum detectable limit is about 1 mg/l sulfate. This method is applicable for potable waters, waters containing small quantities of organic materials and samples have no colors or suspended matter in large amounts.

Equipment:

1. Magnetic stirrer.
2. Photometer: Nephelometer or spectrophotometer for use at 420 nm with a light path of 4 - 5 cm or filter photometer with a violet filter having a maximum near 420 nm and a light path of 4 to 5 cm).
3. Stopwatch.
4. Measuring spoon, capacity 0.2 to 0.3 ml.

Reagents:

1. Buffer solution (A) : 30 g $MgCl_2 .6H_2O$ + 5 g $CH_3COONa.3H_2O$ +1 g KNO_3 + 20 ml CH_3COOH in 500 ml distilled water. The solution is diluted to 1000 ml.
   Or Buffer solution (B): Mix 50 ml glycerol + 30 ml concentrated HCl + 300 ml distilled water +100 ml ethyl alcohol 95% or isopropanol alcohol+ 75 g NaCl
1. Barium chloride, $BaCl_2$, crystals, 20 to 30 meshes.
2. Sodium carbonate solution (approximately 0.05 N): Dry 3 to 5 g of primary standard Na_2CO_3 at 250°C for 4 hours and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1 liter volumetric flask and fill to the mark with distilled water.
3. Standard sulfate solution (1.00 ml ≡ 100 µg SO_4): Dissolve 147.9 mg anhydrous Na_2SO_4 in distilled water in a 1 liter volumetric flask and dilute to the mark with distilled water.

Procedure:

1. Place 100 ml of the test sample or a suitable portion diluted to 100 ml, into a 250 Erlenmeyer flask, add exactly 20 ml of Buffer solution (A) or 5 ml of buffer solution (B) and mix.
2. While the solution is being stirred, add a measuring spoonful of $BaCl_2$ crystals and begin timing immediately. Stir exactly 1.0 minute at a constant speed. Pour the solution into the absorbance cell and continuously measure the turbidity every 30 second intervals for 4 minutes.

3. Record the maximum reading obtained in the 4 minute period.
4. Prepare a calibration curve using standard sulfate solution (0 - 40 mg/l sulfate).

Calculation:

Read mg SO_4 from the calibration curve:

$$SO_4\ , (mg/l) = \frac{mg\ SO_4 \times 1000}{ml\ sample}$$

Interferences:

1. Color or suspended matter in large amounts interferes. Some suspended matter may be removed by filtration, if both are small in comparison with the SO_4^{2-} concentration. Silica in excess of 500 mg/l interferes. Waters containing large quantities of organic materials affect precipitation of $BaSO_4$ satisfactorily.
2. In potable waters, there are no ions other than SO_4^{2-} that will form insoluble compounds with barium under strongly acid conditions. Make determination at room temperature; variation over a range of 10 °C will not cause appreciable error.
3. Positive interference is caused by silicate and arsenate, only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate.
4. Blue color is caused by ferrous iron, if present, but this does not affect the results if ferrous iron concentration is less than 100 mg/l. Sulfide interference may be overcome by oxidation with bromine water.

References:

- USEPA “Methods for Chemical Analysis of Water and Wastes”, Ohio, 1983 (Method 375.4).
- “Standard Methods for Examination of Water and Wastewater”, 23rd Edition, American Public Health Association (APHA), Washington 2017(Method 4500-SO_4 - E).

(II.12) Sulfide

(Spectrophotometry - DMPD Method)

Principle:

Sulfide ion reacts with dimethyl-*p*-phenylenediamine (*p*-aminodimethyl aniline) (DMPD) in the presence of ferric chloride to produce methylene blue, a dye which is measured at a wavelength maximum of 625 nm. The method is suitable for the measurement of sulfide in concentrations up to 20 mg/l. Preservation of sulfide in samples collected in the field during sampling is performed by addition of 4 drops of 2 N zinc acetate to 100 ml of an alkaline sample (pH > 9).

Equipment:

1. Matched test tubes, approximately 125 mm long and 15 mm outer diameter.
2. Droppers, delivering 20 drops/ml. To obtain uniform drops, hold dropper in vertical position and allow drops to fall slowly.
3. Spectrophotometer, for use at 625 nm with cells of 1.0 cm and 10.0 cm light path or filter photometer, with filter providing transmittance near 625 nm.

Reagents:

1. Amino-sulfuric acid stock solution: Dissolve 27 g N, N-dimethyl-*p*-phenylene-diamine oxalate (*p*-aminodimethyl-aniline) in a cold mixture of 50 ml concentrated H_2SO_4 and 20 ml distilled water in a 100 ml volumetric flask. Cool and dilute to the mark. If the solution color becomes dark, discard and purchase fresh reagent. Store in dark glass bottle.
2. Amino-sulfuric acid reagent: Dilute 25 ml amino-sulfuric acid stock solution with 975 ml of (1+1) H_2SO_4. Store in a dark glass bottle. This solution should be clear.
3. Ferric chloride solution: Dissolve 100 g $FeCl_3.6H_2O$ in 40 ml distilled water.
4. Sulfuric acid solution, H_2SO_4 (1+1).
5. Diammonium hydrogen phosphate solution: Dissolve 400 g $(NH_4)_2HPO_4$ in 800 ml distilled water.
6. Methylene blue solution (I): Dissolve 1.0 g of methylene blue in distilled water in a 1 liter volumetric flask and dilute to the mark. The dye content reported on the label should be 84% or more. Standardize against sulfide solutions of known strength and adjust concentration so that 0.05 ml (1 drop) equals 1.0 mg/l sulfide.
7. Ethylene blue solution (II): Dilute 10.0 ml of adjusted ethylene blue solution (I) to 100 ml with distilled water in a volumetric flask.
8. Standardization of methylene blue solution:
   - Place several grams of clean, washed crystals of sodium sulfide $Na_2S.9H_2O$ in a small beaker. Add enough water to cover the crystals.

- Stir occasionally for few minutes. Pour the solution into another vessel. This reacts slowly with oxygen but the change is insignificant over a few hours. Make the solution daily.
- To 1 liter of distilled water, add 1 drop of sulfide solution and mix. Immediately determine the sulfide concentration by methylene blue procedure and by the titrimetric iodide procedure.
- Repeat using more than one drop of sulfide solution or less water until at least five tests have been made in the range of 1 to 8 mg/l sulfide.
- Calculate the average percent error of the methylene blue procedure as compared to the titrimetric iodide procedure.
- Adjust by dilution or by adding more dye to methylene blue solution (I).

Procedure:

A. Color development:

1. Transfer 7.5 ml of sulfide containing sample to each of two matched test tubes (A) and (B), using a special wide tipped pipet or filling to a mark on the test tubes.
2. To tube (A), add 0.5 ml amine-sulfuric acid reagent and 0.15 ml (3 drops) $FeCl_3$ solution.
3. Mix immediately by inverting the tube only once.
4. To tube (B), add 0.5 ml of (1+1) H_2SO_4 and 0.15 ml (3 drops) $FeCl_3$ solution and mix.
5. Color will develop in tube (A) in the presence of sulfide. Color development is usually complete in about 1 minute, but a longer time is often required for the fading of the initial pink color.
6. Wait 3 to 5 minutes, and add 1.6 ml $(NH_4)_2HPO_4$ solution to each tube.
7. Wait 3 to 5 minutes and make color comparisons. If zinc acetate was used wait at least 10 minutes before making comparison.

B. Color comparison:

1. Use a 1.0 cm cell for 0.1 to 2.0 mg sulfide /l and 10.0 cm cell for up to 20 mg/l.
2. Zero instrument with portion of sample from tube (B).
3. Prepare a calibration curve from data obtained in methylene blue standardization, plotting concentration obtained from titrimetric iodide procedure versus absorbance. A linear relationship can be assumed from 0 to 1.0 mg/l. Read the sulfide concentration from the calibration curve.

Calculation:

$$\text{Sulfide, (mg/l)} = \frac{\text{Reading graph, mg x 1000}}{\text{Volume of sample (ml)}}$$

Precautions:

1. Samples must be taken with a minimum aeration. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide into an un-measurable form. Dissolved oxygen should not be present in any water used for dilution of the standards.
2. The analysis must be started immediately.
3. Color and turbidity may interfere.

Interferences:

Strong reducing agents interfere in the methylene blue method by preventing formation of the blue color. Thiosulfate at concentration of 10 mg/l may retard color formation or completely prevent it. Ferrocyanide produces a blue color. Eliminate interferences due to sulfite, thiosulfate, iodide and many others soluble substances but not ferrocyanide by first precipitating ZnS removing the supernatant and replacing it by distilled water.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 376.2).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-S (D)].

(II.13) Sulfide

(Iodo - Titrimetry)

Principe:

Sulfide is determined by oxidation to sulfur by adding a known excess amount of iodine followed by titration of the unreacted iodine with standard sodium thiosulfate solution. The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds.

Equipment:

1. General laboratory glasswares.
2. Filter paper.

Reagents:

1. HCl, 6 N.
2. Standard iodine solution, 0.025 N:
3. Dissolve 20 to 25 g KI in a little water and add 3.2 g iodine. After iodine has dissolved, dilute to 1000 ml and standardize against (0.025 N) $Na_2S_2O_3$ using starch solution as indicator.
4. Standard sodium thiosulfate solution 0.025 N: Dissolve 6.205 g $Na_2S_2O_3.5H_2O$ in distilled water, add 0.4 g solid NaOH or 1.5 ml 6 N NaOH solution and dilute to 1000 ml.
5. Starch solution: Use either an aqueous solution or soluble starch powder mixtures.
6. $K_2Cr_2O_7$ for $Na_2S_2O_3$ standardization.

Standardization of sodium thiosulfate $Na_2S_2O_3$ solution, 0.025 N:

1. Dissolve 24.812 g $Na_2S_2O_3.5H_2O$, in distilled water and complete to 1 litert to give (0.1 N) solution. Standardize every 2 weeks.
2. Dissolve 4.904 g $K_2Cr_2O_7$, in 1 liter of distilled water to give 0.1 N solution.
3. Take 80 ml of deionized water, add 1 ml of concentrated. H_2SO_4, 10 ml of (0.1 N) $K_2Cr_2O_7$, and 1 g of KI. Let the mix 6 minutes in the dark, the color becomes yellow.
4. Titrate with $Na_2S_2O_3$ (0.025 N) until yellow color is discharged, then add starch indicator and titrate until blue color disappear (colorless).

$$N = (\text{ml } K_2Cr_2O_7 \times N\ K_2Cr_2O_7)/ \text{ml } Na_2S_2O_3 \text{ consumed.}$$

Procedure:

1. Transfere a 100 ml aliquot of the test sample to a 500 ml flask.
2. Add 1 ml of 6 N HCl, and a suitable amount of iodine (should be known). If the iodine color disappears, add more iodine till the color remain, record the volume of iodine used. Titrate with reducing solution (0.025 N of $Na_2S_2O_3$) till reach pale yellow color.
3. Then add starch indicator and complete titration till the color becomes blue.

4. Record the milliliters of 0.025N $Na_2S_2O_3$ used.
5. If sulfide was precipitated with zinc, ZnS should be filtered out. Return the filtrate with the precipitate to the origin bottle and add 100 ml water. Add iodine solution and HCl, and titrate.

Calculation:

1.0 ml (0.025 N) iodine solution reacts with 0.4 mg S^{2-}

$$\text{mg } S^{2-}/l = \frac{(A \times B) - (C \times D) \times 16000}{\text{volume of sample (ml)}}$$

where:

A = ml iodine solution.

B = normality of iodine solution.

C = ml $Na_2S_2O_3$ solution.

D = normality of $Na_2S_2O_3$.

16 = equivalent weight of sulfide.

1000 = conversion factor (ml/ l).

Precautions:

1. If the sample is not preserved, the analysis must be started immediately.
2. Samples must be takes with minimum aeration. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide to an immeasurable form.

Interferences:

1. The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds.
2. Interferences due to sulfite, thiosulfate, iodide, and many other soluble substances, but not ferrocyanide, are eliminated by first precipitating ZnS, removing the supernatant, and replacing it with distilled water.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-S^{2-} (F)]).

(II.14) Phosphorus (Orthophosphate)

(Spectrophotometry - Vanadomolybdophosphate Method)

Principle:

Orthophosphate in the presence of molybdate and vanadate, under acid conditions forms a yellow vanadomolybdo-phosphoric acid. The intensity of the yellow color is proportional to phosphate concentration. The minimum detectable concentration is 0.2 mg phosphorus/l in 1.0 cm spectrophotometric cell (cuvette).

Equipment:

1. Spectrophotometer for use at 400-490 nm.
2. Acid-washed glassware.
3. Filtration apparatus and filter paper.

Reagents:

1. Phenolphthalein indicator.
2. Hydrochloric acid (1:1).
3. Activated carbon.
4. Vanadate-molybdate reagent:
   - Solution (I): Dissolve 25 g ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 300 ml distilled water.
   - Solution (II): Dissolve 1.25 g ammonium metavanadate NH_4VO_3 by heating to boiling in 300 ml distilled water. Cool and add 330 ml of concentrated HCl. Cool solution (II) to room temperature pour solution (I) into solution (II), mix and dilute to 1 liter.
5. Standard phosphate solution: Dissolve 219.5 mg of anhydrous KH_2PO_4 in distilled water and dilute to 1000 ml (1.00 ml ≡ 50 μg PO_4-P).

Procedure:

1. If sample pH is greater than 10, add 0.05 ml (1 drop) phenolphthalein indicator to 50 ml sample and discharge the red color with (1:1) HCl before diluting to 100 ml.
2. Remove excessive color in the sample by shaking about 50 ml with 200 mg activated carbon in an Erlenmeyer flask for 5 min, and filter to remove carbon.
3. Place 35 ml or less of sample containing 0.05 - 1.0 mg P, in a 50 ml volumetric flask. Add 10 ml vanadate-molybdate reagent and dilute to the mark with distilled water.
4. Prepare a blank in which 35 ml distilled water is substituted for the sample.
5. After 10 min, measure the absorbance and compare with the calibration curve.
6. Prepare a calibration curve by using 1-20 μg P/sample versus a blank at a wavelength of 400-490 nm, and compare with a calibration standard phosphate solution and proceed as above.

Calculation:

$$\text{Phosphate-P, (mg/l)} = \frac{\text{mg P (in 50 ml final volume) x 1000}}{\text{Volume of sample (ml)}}$$

Precautions*:*

1. Positive interference is caused by silicate and arsenate, only if the sample is heated.
2. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate.
3. Blue color is caused by ferrous iron concentration but this does not affect results if ferrous concentration is less than 100 mg/l.
4. Sulfide interference may be removed by oxidation with bromine water.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-P (C).

(II.15) Phosphorus, Orthophosphate

(Spectrophotometry - Molybdenum Blue Method)

Principle:

Phosphate ions react with ammonium molybdate to form molybdophosphoric acid which upon reduction with stannous chloride gives intense blue colored molybdenum blue. This method is more sensitive than Method (II.14). It allows measurements of down to 7 µg P/l by use of increased light path length. Below 100 µg P/l, an extraction step may increase reliability and reduce interference. The minimum detectable concentration is about 3 mg P/l. The sensitivity at 0.3010 absorbance is about 10 µg P/l for an absorbance change of 0.009.

Equipment:

The same apparatus is required as for "Method II. 14", except that a pipetting bulb is required for the extraction step. Set spectrophotometer at 625 nm in the measurement of benzene-isobutanol extracts and at 690 nm for aqueous solutions. If the instrument is not equipped to read at 690 nm, use a wavelength of 650 nm for aqueous solutions, with somewhat reduced sensitivity and precision.

Reagents:

1. Phenolphthalein indicator, aqueous solution.
2. Ammonium molybdate reagent (I): Dissolves 25 g $(NH_4)_6MO_7O_{24}.4H_2O$ in 175 ml distilled water. Cautiously add 28 ml of concentrated H_2SO_4 to 400 ml distilled water. Cool, add molybdate solution, and dilute to 1 liter.
3. Stannous chloride reagent (I)*:* Dissolve 2.5 g fresh $SnCl_2.2H_2O$ in 100 ml glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.
4. Standard phosphate solution.
5. Reagents for extraction:
   - Benzene-isobutanol solvent: Mix equal volumes of benzene and isobutyl alcohol. (Caution: This solvent mixture is highly flammable).
   - Ammonium molybdate reagent (II): Dissolve 40.1 g $(NH_4)_6MO_7O_{24}.4H_2O$ in approximately 500 ml distilled water. Slowly add 396 ml ammonium molybdate reagent (I). Cool and dilute to 1 liter.
   - Alcoholic sulfuric acid solution: Cautiously add 20 ml of concentrated H_2SO_4 to 980 ml methyl alcohol with continuous mixing.
   - Dilute stannous chloride reagent (II): Mix 8 ml of stannous chloride reagent (I) with 50 ml glycerol. This reagent is stable for at least 6 months.

Procedure:

1. Preliminary sample treatment: To 100 ml sample containing not more than 200 mg P and free from color and turbidity, add 0.05 ml (1 drop) phenolphthalein indicator. If sample turns pink, add strong acid solution dropwise to discharge the color. If more than 0.25 ml (5 drops) is required, take a smaller sample and dilute to 100 ml with distilled water after first discharging the pink color with acid.
2. Color development: Add, with thorough mixing after each addition, 4.0 ml molybdate reagent (I) and 0.5 ml (10 drops) stannous chloride reagent (I). Rate of color development and intensity of color depend on the temperature of the final solution, each 1 ºC increase producing about 1 % increase in color. Hold samples, standards, and reagents within 2 ºC in the temperature range between 20 and 30 ºC.
3. Color measurement: The color is measured after 10 min, but before 12 min. Use the same specific interval for all determinations, measure the developed color photometrically at 690 nm and compare with a calibration curve, using a distilled water blank. Light path lengths suitable for various concentration ranges are given in Table (II.3).

Table (II.3) Selected light path lengths

Approximate P range, mg/l	Light path, cm
0.3-2	0.5
0.1-1	2
0.007-0.2	10

4. Always run a blank on the used reagents and distilled water. Because the color at first develops progressively and later fades, maintain equal timing conditions for samples and standards. Prepare at least one standard with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0-mg/l range.

Extraction:

When increased sensitivity is desired or interferences must be overcome, extract phosphate as follows:

1. Pipet a 40-ml sample or diluted sample to that volume, into a 125-ml separatory funnel. Add 50.0 ml benzene-isobutanol solvent and 15.0 ml molybdate reagent (II).
2. Close funnel at once and shake vigorously for exactly 15 s. If condensed phosphate is present, any delay will increase its conversion to orthophosphate. Remove stopper and withdraw 25.0 ml of separated organic layer, using a pipette with safety bulb.
3. Transfer to a 50-ml volumetric flask, add 15 to 16 ml alcoholic H_2SO_4 solution, swirl, add 0.50 ml (10 drops) dilute stannous chloride reagent (II), swirl, dilute to the mark with alcoholic H_2SO_4 and mix thoroughly.

4. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare a blank by carrying 40 ml distilled water through the same procedure used for the sample.
5. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

Calculation:

Calculate phosphorus content as follows:

1. Direct procedure:

$$\text{mg P/l} = \frac{\text{mg P (in 104.5 ml final volume) x 1000}}{\text{Volume of sample (ml)}}$$

2. Extraction procedure:

$$\text{mg P/l} = \frac{\text{mg P (in 50 ml final volume) x 1000}}{\text{Volume of sample (ml)}}$$

Precautions:

1. Positive interference is caused by silicate and arsenate, only if the sample is heated.
2. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate.
3. Blue color is caused by ferrous iron concentration but this does not affect results if ferrous concentration is less than 100 mg/l.
4. Sulfide interference may be removed by oxidation with bromine water.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-P (C)

(II.16) Cyanide, Total

(Spectrophotometry - Pyridine-Barbituric Acid Method)

Principle:

Cyanide as hydrocyanic acid (HCN) is released from cyanide complexes by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined colorimetrically or potentiometrically using a cyanide ion selective electrode. In the colorimetric measurement, the cyanide is converted into cyanogen chloride, CNCl, and react with pyridine-pyrazolone or pyridine-barbituric acid reagent. The absorbance is read at 620 nm when using pyridine-pyrazolone or 578 nm for pyridine-barbituric acid. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards. The method is used for determining cyanide concentrations below 1 mg/l and is sensitive to about 0.02 mg/l.

Equipment:

1. Reflux distillation apparatus such as shown in Figure (II.1). A 1 liter boiling flask with inlet tube and provision for condenser is used.
2. Spectrophotometer suitable for measurements at 578 nm or 620 nm with a 1.0 cm cell or larger.
3. Reflux distillation apparatus for sulfide removal is shown in Figure (II.2). The air inlet tube should not be fritted. The cyanide absorption vessel should be the same as the sulfide scrubber. The air inlet tube should be fritted.
4. Flow meter.

Reagents:

1. Sodium hydroxide solution, 1.25 N: Dissolve 50 g of NaOH in distilled water, and dilute to 1 liter with distilled water.
2. Lead acetate: Dissolve 30 g of $Pb(C_2H_3O_2).3H_2O$ in 950 ml of distilled water. Adjust the pH to 4.5 with acetic acid and dilute to 1 liter.
3. Sulfuric acid; 18 N: Slowly add 500 ml of concentrated H_2SO_4 to 500 ml of distilled water.
4. Sodium dihydrogen phosphate, 1 M: Dissolve 138 g of $NaH_2PO_4.H_2O$ in 1 liter of distilled water and refrigerate.
5. Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g KOH in 900 ml of distilled water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration so that (1.0 ml ≡ 1.0 mg CN).
6. Standard cyanide solution, intermediate: Dilute 100.0 ml of stock (1.0 ml ≡ 1.0 mg CN) to 1000 ml with distilled water (1.0 ml ≡ 100.0 μg).

7. Working standard cyanide solution: Daily prepare fresh cyanide solution by diluting 100.0 ml of intermediate cyanide solution to 1000 ml with distilled water and store in a glass stoppered bottle (1.0 ml ≡ 10.0 µg CN).
8. Standard silver nitrate solution, 0.0192 N: Crush approximately 5 g $AgNO_3$ crystals and dry to a constant weight at 40 °C. Weigh out 3.265 g of dried $AgNO_3$, dissolve in distilled water, and dilute to 1000 ml (1.0 ml ≡ 1.0 mg CN).
9. Rhodanine indicator: Dissolve 20 mg of *p*-dimethyl-amino-benzalrhodanine in 100 ml of acetone.
10. Chloramine-T solution: Dissolve 1.0 g of white, water soluble chloramine-T in 100 ml of distilled water and refrigerate until ready to use. The solution is prepared fresh daily.
11. Color reagent (Pyridine-Barbituric Acid Reagent): Place 15 g of barbituric acid in a 250 ml volumetric flask and add just enough distilled water to wash the sides of the flask and wet barbituric acid. Add 15 ml of concentrated HCl, mix, and cool to room temperature. Dilute to 250 ml with distilled water and mix. This reagent is stable for approximately six months if stored in a cool, dark place.
12. Magnesium chloride solution: Weigh 510 g of $MgCl_2.6H_2O$ into a 1000 ml flask, dissolve and dilute to the mark with distilled water.
13. Sulfamic acid, H_3NSO_3.

Procedure:

A. For sulfide free samples:

1. Place 500 ml of cyanide containing test sample or an aliquot diluted to 500 ml in 1 liter boiling flask.
2. Pipet 50 ml of sodium hydroxide into the absorbing tube. Connect the boiling flask, condenser, absorber and trap in the train.
3. Start a slow stream of air entering the boiling flask by adjusting the vacuum source.
4. Adjust the vacuum so that approximately 2 bubbles of air per second enter the boiling flask through the air inlet tube.

B. For samples containing sulfide:

1. Place 500 ml of the test sample or an aliquot diluted to 500 ml in 1 liter boiling flask. Pipet 50 ml of sodium hydroxide into the absorbing tube. Add 25 ml of lead acetate to the sulfide scrubber. Connect the boiling flask, condenser, scrubber and absorber in the train. The flow meter is connected to the outlet tube of the cyanide absorber.
2. Start a stream of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that approximately 1.5 liters per minute enters the boiling flask through the air inlet tube. The bubble rate may not remain constant while heat is being applied to the flask. It may be necessary to re-adjust the air rate occasionally.

C. For samples containing nitrate and/or nitrite:

1. Add 2 g of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 minutes prior to addition of H_2SO_4.
2. Slowly add 5 ml 18 N sulfuric acid through the air inlet tube. Rinse the tube with distilled water and allow the air-flow to mix the flask contents for 3 minutes. Pour 20 ml of magnesium chloride into the air inlet and wash down with a stream of water.
3. Heat the solution to boiling reflux for one hour. Turn off heat and continue the air-flow for at least 15 minutes. After cooling the boiling flask, disconnect absorber and close off the vacuum source.

D. Color development:

1. Drain the solution from the absorber into a 250 ml volumetric flask. Wash the absorber with distilled water and add the washings to the flask. Dilute to the mark with distilled water.
2. Withdraw 50 ml or less of the solution from the flask and transfer to a 100 ml volumetric flask. If less than 50 ml is taken, dilute to 50 ml with 0.25 N sodium hydroxide solutions. Add 15.0 ml of sodium phosphate solution and mix. Add 2 ml of chloramine-T and mix. After 1 to 2 minutes, add 5 ml of pyridine-barbituric acid solution and mix. Dilute to mark with distilled water and mix again. Allow 8 minutes for color development, and read the absorbance at 578 nm in a 1 cm cell within 15 minutes.
3. Prepare a series of standards by pipetting 0 - 20 ml of standard cyanide solution (10 µg/ml) into 250 ml volumetric flasks. To each standard, add 50 ml of 1.25 N sodium hydroxide and dilute to 250 ml with distilled water. Proceed with the analysis and prepare a standard curve by plotting absorbance of standard vs. cyanide concentration.

Calculation*:*

Calculate the cyanide, in µg/l, in the original sample as follows:

$$CN, (\mu g/l) = \frac{A \times 1{,}000}{B} \times \frac{50}{C}$$

where:

A = cyanide content (µg) read from standard curve.

B = volume (ml) of original sample used in distillation.

C = volume (ml) taken for colorimetric analysis.

Precautions:

1. Interferences by sulfides are eliminated by scrubbing in lead acetate.
2. Interferences caused by nitrate and/or nitrite are removed by pretreatment with sulfamic acid.

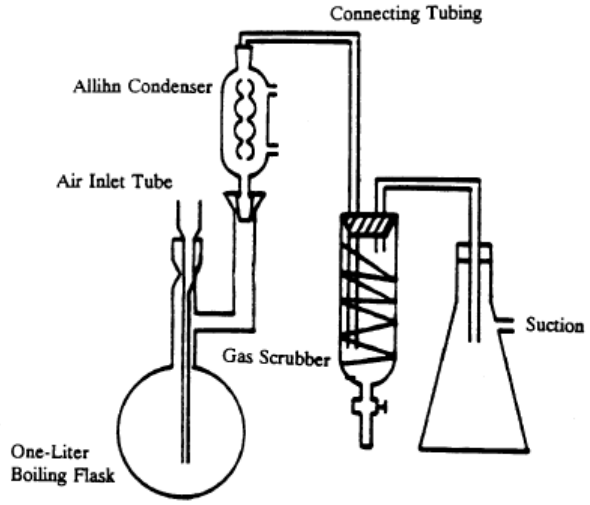
3. Fatty acids are removed by extraction with isooctane, hexane, or chloroform (in the order named) and solvent volume equals to 20 % of sample volume.
4. Analysis must be performed in the hood.

Interferences:

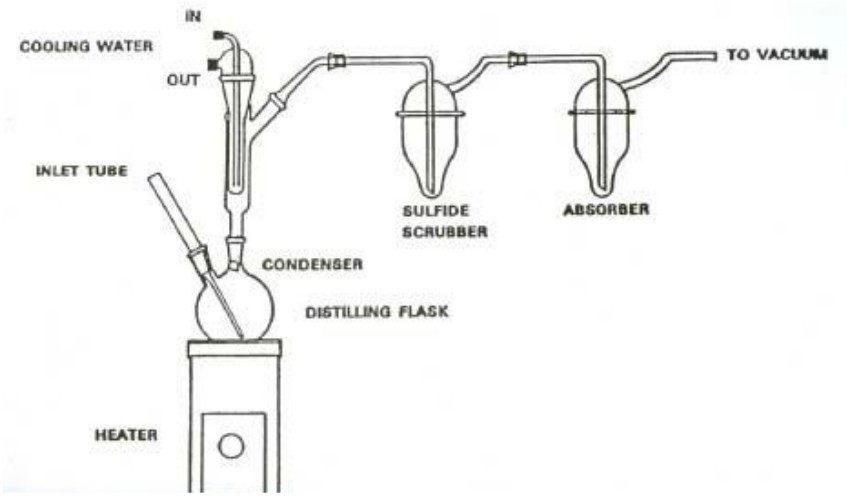
All known interferences are successfully eliminated or reduced to a minimum by distillation.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 335.3).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-CN I]).



(Fig.II.1) Cyanide distillation apparatus



(Fig.II.2) Cyanide distillation apparatus

(II.17) Cyanide, Total

(*Potentiometry-Ion Selective Electrode)*

Principle:

Cyanide ion in the alkaline distillate can be determined by preliminary treatment procedure followed by potentiometric measurement using CN^- selective electrode. The method can be used to determine CN^- concentration in the range of 0.05 to 10 mg CN^-/l.

Equipment:

1. Expanded-scale pH meter.
2. Cyanide-ion selective electrode.
3. Reference electrode, double junction.
4. Magnetic mixer.
5. Koch microburette, 10 ml capacity.

Reagents:

1. Stock standard cyanide solution: dissolve 1.6 g NaOH and 2.51g KCN in 1 liter distilled water. (Caution: KCN is highly toxic) standardize against $AgNO_3$ titrant (1.0 ml ≡ 1.0 mg CN^-).
2. Sodium hydroxide solution: Dissolve 1.6 g NaOH in water and dilute to 1 liter.
3. Standard cyanide solution, intermediate: Dilute 100.0 ml of the stock solution (1.0 ml ≡ 1.0 mg CN) to 1000 ml with distilled water (1.0 ml ≡ 100.0 µg).
4. Working standard cyanide solution: Prepare fresh daily solution by diluting 100.0 ml of intermediate cyanide solution to 1000 ml with distilled water and store in a glass stoppered bottle (1.0 ml ≡ 10.0 µg CN).
5. Potassium nitrate solution: Dissolve 100 g KNO_3 in water, dilute to 1liter and adjust to pH 12 with KOH, this solution is used as outer filling solution for the double junction reference electrode.

Procedure:

1. Prepare four 100 ml working standard solutions containing 2.5, 0.25, 0.125 and 0.025 µg CN^- /ml in NaOH dilution solution.
2. Transfer the first standard solution to 250 ml beaker, immerse CN^- and double. Junction reference electrodes, mix well using a magnetic stirrer.
3. Maintain the same stirring rate for all solutions.
4. After equilibrium is reached, record the potential reading.
5. Repeat steps 2 - 4 for the other standards (always start from the lowest to the highest concentration).
6. Plot log CN^- concentration versus the potential developed in solution.
7. Follow up the manufacture instruction for direct reading of the ion meters.
8. Measurement of sample: Place 100 ml test solution or a portion diluted to 100 ml with NaOH in a 250 ml beaker.

9. Immerse a cyanide ion selective electrode in conjunction with a double junction reference electrode in the test solution. Stir the solution using a magnetic stirrer.
10. After equilibrium is reached, record the potential value indicated on the ion meter or find the concentration from graph prepared as above.

Interferences:

All known interferences are eliminated or reduced to a minimum by distillation.

Calculation:

$$\text{mg } CN^-/l = \frac{A \times 1000 \times 50}{B \times C}$$

where:

A = cyanide content (µg) read from standard curve.

B = volume (ml) of original sample used for distillation.

C = volume (ml) taken for colorimetric analysis.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method [4500-CN (F)]).

(II.18) Cyanide

(Ion-Chromatography)

Principle:

The ion chromatography method is fast and accurate for determining free cyanide in drinking water samples. The method is compatible with the basic solutions used to preserve drinking water samples for cyanide analysis and is unaffected by other compounds typically found in drinking water. This method simultaneously determine cyanide with other ions. Data is recorded, integrated and results reported by computer. Since cyanide is reactive and unstable, drinking water samples should be stabilized and free cyanide measured as soon as possible. Oxidizing agents decompose cyanide and any free cyanide present at neutral pH will volatilize to hydrogen cyanide. Because of these issues, the drinking and surface water samples were stabilized as soon as practical. The municipal and drinking water samples were stabilized within one hour of sampling by addition of a 50 % (w/w) sodium hydroxide solution.

Equipment:

Ion chromatograph, class A pipettes and volumetric flasks “sorted sizes”.

Table (II. 4) Chromatographic operational conditions

Chromatographic conditions	
Column	Ion Pac AS7 Analytical, (4 × 250 mm)
Guard column	Ion Pac AG7 Guard, (4 × 50 mm)
Detection	Electrochemical detector (ED), silver working electrode
Sample loop	25 µl
Pump flow rate	1.0 ml/min
Backpressure:	~1800 psi
Runtime	8 min

Sample collection, preservation and handling:

Drinking water, ground water, surface water, wastewater, and ocean water are treated, if necessary, with powdered lead carbonate to remove sulfide interferences, and with sodium thiosulfate to remove interfering oxidants. The samples are then adjusted with sodium hydroxide to pH = 12.5 and stored in amber bottles at 4±2°C. Samples preserved in this way must be analyzed within 14 days. On the day of analysis, the samples are brought to room temperature, filtered through 0.45-µm pore-size by Whatman filter paper or equivalent filters and transferred to the auto sampler vials.

Glassware cleaning:

In addition to normal cleaning operations, acid wash (10 % hydrochloric acid) glassware used for reagent preparation followed by at least five rinses with deionized water are commonly used. Deionized water: more than 18 MΩ /cm, is used immediately after purification.

Reagents:

1. Chemical purity requirements. To obtain reliable, consistent and accurate results, it is required to use eluents free of ionic impurities. Chemicals, solvents and deionized water used to prepare eluents and standards must be of the highest purity available. Low trace impurities and low particle levels in eluents also protect the ion exchange columns and system components.
2. Eluent solution. It consists of sodium acetate anhydrous, 0.5 M, sodium hydroxide, 0.1M and ethylenediamine, 5% (v/v).
3. Mixed eluent. Dissolve 4.0 g sodium hydroxide and 41.0 g sodium acetate anhydrous with little deionized water in 1000 ml measuring flask, add 5 ml ethylenediamine, and dilute to the mark using deionized water.
4. Stock cyanide standard solutions (1000 mg/l). Dissolve approximately 2 g of sodium hydroxide in approximately 500 ml of reagent water contained in a 1-liter volumetric flask. Add 2.51 g of potassium cyanide, dilute to one liter with reagent water, and mix well. Store KCN solution in an amber glass container in refrigerator at 4±2 °C**.** The stock solution is stable for one month**.**
5. Working cyanide standards. 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5 and 5.0 mg/l are prepared according to Table (II.5).

Table (II.5) Cyanide working standards (mg/l CN) prepared from 10 mg/l CN standard

Concentration used, mg/l	Volume taken, Ml	Final volume, ml	Final concentration, mg/l
1000	1	100	10
10	50	100	5
5	50	100	2.5
2.5	50	100	1.25
1.25	50	100	0.625
0.625	50	100	0.313
0.313	50	100	0.156
0.156	50	100	0.078
0.078	50	100	0.039

N.B.The working standards should be prepared daily.

Procedure:

1. Set-up the chromatograph system according to the equipment manuals.
2. If the concentration of the samples is greater than 15 % of the highest standard, dilute the sample and rerun.

3. For each run, monitor cyanide sensitivities as determined by analysis of calibration standards. Sensitivities should not deviate by more than 10 % from expected values. Check calibration stability by analyzing a check standard (QC sample) every 10-sample tubes and after all the samples.
4. If results are not within 10 % of the expected values, then correct the problem and re-analyses the affected samples.

Quality control:

1. Results for accuracy of the quality control samples must be within supplier designated limits or, where these are not available, results must be within 10 % of expected values.
2. Analyze at least 10 % of the samples in replicate, with a minimum of one sample per set. The % RSD of duplicate results not close to the detection limit must not exceed 20 %.
3. Analyze at least one blank with each batch of samples. Blank results must be less than the detection limits. Investigate occurrences of positive blanks and rerun sample analysis after solving the causing problem.
4. Duplicate sample analyses should be performed on about 5 % of routinely analyzed samples.
5. Analyze a check solution or standard after every ten sample vials. Check the slope of the calibration curve. The correlation coefficient must be greater than 0.99.
6. Create a control chart using the results of the quality control samples (QCS) (See section X). Control charts are an efficient way for analyzing performance data and to evaluate the instrument. Excel program can be used.
7. Method detection limits (MDL). It must be established for all analyses, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. Take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where:

t = student's t value for a 99 % confidence level and a standard deviation estimate with (n-1) degrees of freedom [t = 3.14 for seven replicates].

S = standard deviation of the replicate analyses.

MDL should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response. The cyanide detection limit is 0.005 mg/l.

A. Method performance and validation:

- Precision: Check the relative standard deviation of three replicates (Replicate RSD), it

should be less than 5 %.

- Accuracy: To verify the calibration standards, it is recommended to run QC samples at the beginning and at the end of the run for water samples. Certified reference materials are used and all glassware should be class A. The used bottles should be kept tightly capped and stored under normal laboratory conditions. All the different concentrations are reported in Table (II. 6).

Table (II.6) Accuracy, standard deviation (precision) and uncertainty

Designed value, mg/l	Measurement value	Average value	Accuracy, %	Bias	Standard deviation	Uncertainty	RSD, %
0.100	0.097-0.101	0.0987	98.714	0.000	0.00150	±0.0030	1.516
5.00	5.02 – 5.100	5.06428	101.286	0.000	0.02992	±0.0598	0.591

Data analysis and calculation:

Compute sample concentration by comparing sample response with the standard curve. Multiply the results by appropriate dilution factor. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding this level should be diluted and reanalysed. Record the results as µg/l.

Safety and precautions:

Cyanide is a poison by inhalation, contact, and ingestion. It generates hydrogen cyanide gas in neutral or acidic media. Solutions containing cyanide must be stabilized with a base. Read and follow the material safety sheet (MSS) instructions for handling, exposure, and disposal information. Also consult local safety personnel for regulations concerning the proper disposal of cyanide waste.

Interferences:

1. In the EPA methods, sulfide and sulfide-generating compounds are cited most often as potential interferences. Sulfide complexes with free cyanide to form thiocyanate.
2. Other interferences cited, are nitrate, nitrite, and chlorine. Copper and other transition metals complex interact with cyanide, preventing the measurement of free cyanide.
3. Copper (II) rapidly oxidizes cyanide to cyanogen gas and copper (I) cyanide precipitate.
4. Acidification of cyanide solutions will volatilize cyanide in the form of the high poisonous hydrogen cyanide gas, thus preventing accurate measurement of free cyanide.
5. When cyanide electrode is used for measurement, iodide, thiosulfate, bromide, thiocyanate, and sulfide are potential inferences.

References:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-CN

(B).

- Giuriati, C., Cavalli, S., Gorni, A., Badocco, D., Pastore, P. (2004): Ion Chromatographic Determination of Sulfide and Cyanide in Real Matrices by Using Pulsed Amperometric Detection on a Silver Electrode. J. Chromatogr. A 2004, 1023, 105–112.

(II.19) Fluoride, Chloride, Bromide, Nitrite, Nitrate, Phosphate and Sulfate

(Ion-Chromatography)

Principle:

This method simultaneously determines fluoride, chloride, bromide, nitrite, nitrate, phosphate and sulfate by using ion chromatography. Ion chromatography system is used and data is recorded, integrated and results reported by a computer. This automated method is applicable for drinking, surface, domestic and industrial waste waters.

Equipment:

Ion chromatograph system equipped with the following components:

1. Detector set to conductivity mode.
2. Analytical column.
3. Guard column.
4. An injection loop (~25 µl).
5. Anion self-regenerating suppresser column (4-mm).
6. Auto sampler tray, auto sampler tubes (5 ml or equivalent) and auto sampler tube caps. The sample tubes are soaked in 10 % hydrochloric acid for at least 15 minutes and rinse at least five times with reagent water before use.
7. Isocratic pump or gradient pump (maximum operating pressure 3000 Psi).

Sample collection, handling and preservation:

1. Collect samples in plastic containers.
2. Store samples in a refrigerator at 4±2 °C.
3. Allow samples to warm to room temperature, filter before analysis and adjust the pH between 0 and 12.
4. The minimum quantity required to analyze one sample is 100 ml.
5. Analysis must be done as soon as possible.
6. Holding times are 48 hours for nitrite, nitrate and phosphate and 28 days for bromide, chloride, fluoride, and sulfate.

Reagents:

A. Stock individual standards:

1. Fluoride standard, 1000 mg/l as F^-
   - Sodium fluoride …….. 2.210 g
   - Deionized water. …….. 1000 ml

Preparation:

- Pre-dry approximately 3 g of sodium fluoride at 104±1°C for two hours, and allow to cool to room temperature in a desiccator for one hour.

- Place 2.210±0.001 g of the pre-dried sodium fluoride into a one liter volumetric flask and dilute to volume with deionized water, and mix.
- Remake stock standard, this solution is stable for at least one month. Store in an amber plastic 1-litre bottle.

2. Chloride standard, 1000 mg/l as Cl^-
   - Sodium chloride (NaCl) …….. 1.6485 g
   - Deionized water …….. 1000 ml

Preparation*:*

- Pre-dry approximately 2 g of sodium chloride at 104±1°C for two hours, and then allow cooling to room temperature in desiccators for one hour.
- Place 1.6485±0.0005 g of the pre-dried sodium chloride into a one litre volumetric flask,
- Dilute to volume with deionized water, and mix.
- Remake stock standard at least every month.

3. Nitrite standard, 1000 mg/l as NO_2^-
   - Sodium nitrite ($NaNO_2$) …….. 1.50 g
   - Deionized water …….. 1000 ml

Preparation:

- Place approximately 800 ml of deionized water into a 1 litre volumetric flask.
- Add 1.50± 0.001 g of sodium nitrite (do not pre-dry) and dissolve.
- Dilute to volume with deionized water, mix and transfer to an amber 1 liter plastic bottle.
- Remake stock standard at least every one month.

4. Bromide standard, 1000 mg/l as Br^-
   - Sodium bromide (NaBr) ……. 1.2877 g
   - Deionized water ……..….. 1000 ml

Preparation*:*

- Pre-dry approximately 2 g of sodium bromide at 104±1 °C for two hours, and allow cooling to room temperature in a desiccatorfor one hour.
- Place 1.2877±0.0005 g of the pre-dried sodium bromide into a one litre volumetric flask.
- Dilute to volume with deionized water, and mix.
- Remake stock standard at least every one month.

5. Nitrate standard, 1000 mg/l as NO_3^-
   - Sodium nitrate ($NaNO_3$) …….. 1.3708 g.
   - Deionized water ……. 1000 ml.

Preparation*:*

- Place approximately 800 ml of deionized water into a 1 liter volumetric flask.
- Add 1.3708±0.001 g of sodium nitrate (do not pre-dry) and dissolve.
- Dilute to volume with deionized water.
- Mix and transfer to an amber plastic 1-litre bottle.
- Remake stock standard at least every one month.

6. Phosphate standard, 1000 mg/l as PO_4^{3-}

-Potassium dihydrogen phosphate (KH_2PO_4) …1.4325 g.

- Deionized water ……1000 ml.

Preparation:

- Pre-dry approximately 5 g of potassium dihydrogen phosphate at 104±1°C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 1.4325 ±0.001g of the pre-dried potassium dihydrogen phosphate into a one litre volumetric flask.
- Dilute to volume with deionized water, and mix.
- Remake stock standard at least every one month.

7. Sulfate standard, 1000 mg/l as SO_4^{2-}

- Sodium sulfate (Na_2SO_4) ……... 1.478 g.
- Deionized water ……..…….. 1000 ml.

Preparation:

- Pre-dry approximately 2 g of sodium sulfate at 104±1 °C for two hours, and allow for cooling to room temperature in a desiccator for one hour.
- Place 1.478±0.001 g of the pre-dried sodium sulfate into a one litre volumetric flask.
- Dilute to volume with reagent water.

B. Mixed stock standards:

Table (II.7) Volumes of the mixed anion stock solutions

No	Standard	Added, ml	Concentration in 500 ml
1	Fluoride	١	2 mg/l
2	Chloride	٤٠	80 mg/l
3	Nitrite	٨	16 mg/l
4	Bromide	٨	16 mg/l
5	Nitrate	٨	16 mg/l
6	Phosphate	١٠	20 mg/l
7	Sulfate	٥٠	100 mg/l

From the individual stock standards, pipette the following quantities into a 500 ml volumetric flask as shown in Table (II. 7). Make to volume with deionized water. Transfer to a 1000 ml plastic bottle and store in a refrigerator at 4±2 °C. Shelf life: 90 days.

C. Working standards:

Three working standards are necessary for calibration of the instrument. Start by making standards No (3), standard No (2) is made from standard No (3), and standard No (1) is made from standard No (3). Pipet 50 ml from standard No (3) and complete to 100 ml using reagent water to make standard No (2), and pipet 25 ml from standard No (3) and complet to 100 ml using deionized water to make standard No (1).

Table (II.8) Volumes of working standards

No	Standard No	Mixed stock, ml	Volumetric flask, (ml)
1	3	-	500
2	2	50	100
3	1	25	100

Table (II. 9) Final concentrations of the working standards

No	Analyte	Standard No (1)	Standard No (2)	Standard No (3)
1	Fluoride	0.5	1.0	2.0
2	Chloride	20	40	80
3	Nitrite	4	8	16
4	Bromide	4	8	16
5	Nitrate	4	8	16
6	Phosphate	5	10	20
7	Sulfate	25	50	100

The solutions should be prepared fresh daily.

D. Stock sodium carbonate eluent solution:

Dissolve 26.49 g of Na_2CO_3 in 400 ml of deionized water (do not pre-dry), dilute to a final volume of 500 ml and store in a refrigerator at 4±2°C. Remake stock once every 3 months.

E. Stock sodium bicarbonate eluent solution:

Dissolve 21.0 g of $NaHCO_3$ in 400 ml of deionize water (do not pre-dry) and dilute to a final volume of 500 ml and store in a refrigerator at 4±2°C. Remake stock once every 3 months.

F. Stock eluent solution for use, (3.5 mM Na_2CO_3/1.0 mM $NaHCO_3$):

Pipet 7.0 ml of 0.5 mM Na_2CO_3 plus 2.0 ml of 0.5 mM $NaHCO_3$ of the concentrate eluent into one liter volumetric flask and dilute to the mark. Prepare enough solution for analysis run and, if necessary, for overnight, based on a pump rate of 1.2 ml/min. (Two litres of eluent is enough for a 25 hour run overnight). The solution is prepared fresh daily.

Procedure:

1. Set-up to a clean column refers to the appropriate column instruction manual. Using the "Schedule Editor" of the chromatograph software, open the appropriate schedule file (with SCH extension).
2. The analytical method file name is "Anions Method" (for AS14 Analytical column). Run the chromatograph software and from the "Run Program" load the method.
3. Run a test standard before beginning the main run in order to condition the instrument and ensure that the instrument is properly set up.
4. Check the retention times for each analyte. A reduced retention time is an indication of a dirty column. If the reduced retention time is not pronounced, change the method to account for new retention times.
5. Edit and fill-in the "Autosampler Table" as needed. Save and print the file.
6. The "Anions.SCH" file may be used as a template for other schedules. Transfer standards (starting with the low standard, St. No.1), QC's (St. No. 2), high standard (St. No. 3) and samples to acid washed 5 ml Autosampler tubes and cap each with an autosampler tube cap. Rinse each tube three times with the test sample. If the samples contain particulate then use a 20 ml glass syringe and 0.5 µm filters to filter the sample into the tubes
7. From the run program load the previously completed "Schedule File" and start this schedule. If the concentration of an analyte is greater than 15 % of the highest standard, dilute the sample and rerun.
8. Sensitivities should not deviate by more than 10 % from expected values. Check calibration stability by analysing a check standard every 11 sample tube and after all the samples. If results are not within 10 % of the expected values, correct the problem and re-analyse the affected samples.

Table (II.10) Detection limit of anions

Parameter	Detection limit, (mg/l)
Fluoride	0.05
Chloride	0.2
Nitrite	0.2
Bromide	0.2
Nitrate	0.2
Phosphate	0.2
Sulfate	0.5

Calculation:

1. The computer performs all calculations and results are printed out. Dilutions should be taken into account in the auto sampler schedule. Run the batch program from the main menu, import the schedule and export as a file.

Quality assurance / quality control:

1. Results for accuracy obtained by quality control samples must be within supplier designated limits. The obtained results must be within 10 % of the expected values.
2. Analyse at least 10 % of the samples in replicate, with a minimum of one sample per set. The % RSD of duplicate results not close to the detection limit must not exceed 20 %.
3. Analyse a spike sample for every 10 samples and at least one per set. Spike recoveries must be within 80 to 120 %.
4. Analyse at least one blank with each batch of samples. Blank results must be less than detection limits. Investigate occurrences of positive blanks and rerun sample analysis after solving the problem.
5. Analyse a check solution or standard after every 10 samples. Check the slope of the calibration curve. The correlation coefficient must be greater than 0.99.

Precautions:

In addition to normal cleaning operations, acid wash (10% hydrochloric acid) glassware used for reagent preparation followed by at least five rinses with deionized water should be conducted. The eluents should be free from ionic impurities and made from high purity chemicals to protect the ion exchange columns and system components.

Method validation:

Table (II.11) Method precision and accuracy

Parameter	Level, mg/l	Average, mg/l
Fluoride	2.0	1.99
Chloride	80	79.8
Nitrite	16	15.6
Bromide	16	16.0
Nitrate	20	16.2
Phosphate	20	20
Sulfate	100	100.1

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 4110, Determination of Anions by Ion Chromatography).

(III) Metal Constituents

(III.1) Manganese

(Spectrophotometry - Persulfate Method)

Principle:

Persulfate oxidation of soluble manganese (II) compounds gives permanganate ions (Mn^{7+}). The reaction is catalyzed by silver nitrate. The colored permanganate is spectrophotometrically measured at 525 nm.

Equipment:

A spectrophotometer.

Reagents:

1. Special reagent. Dissolve 75 g of $HgSO_4$ in 400 ml concentrated HNO_3 and 200 ml distilled water. Add 200 ml 85 % phosphoric acid (H_3PO_4) and 35 mg silver nitrate ($AgNO_3$). Dilute the cooled solution to 1 liter.
2. Ammonium persulfate. Solid $(NH_4)_2S_2O_8$.
3. Standard manganese solution. Prepare a 0.1N potassium permanganate ($KMnO_4$) solution by dissolving 3.2 g $KMnO_4$ in 1 liter distilled water. Heat for several hours near the boiling point (1.0 ml ≡ 50 µg Mn).

Procedure:

1. To a suitable sample portion, add 5 ml of the special reagent and 1 drop H_2O_2.
2. Concentrate to 90 ml by boiling, then add 1 g $(NH_4)_2S_2O_8$.
3. Boil for 1 min, then cool under the tap.
4. Dilute to 100 ml with distilled water.
5. Prepare serial dilutions from the standard.
6. Measure the developed color photometrically at 525 nm and compare with a calibration curve using distilled water blank.

Calculation:

$$\text{Mn, mg/l} = \frac{\mu\text{g Mn in 100 ml final volume}}{\text{ml sample}}$$

When a portion of the digested sample (100 ml final volume) is taken for analysis:

$$\text{Mn, mg/l} = \frac{\mu\text{g Mn/100 ml}}{\text{ml sample}} \times \frac{\times 100}{\text{ml portion}}$$

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 3500-Mn D, p. 3–77).
-

(III.2) Iron

(Spectrophotometry - Phenanthroline Method)

Principle:

On exposure to air or addition of oxidants, ferrous iron (Fe^{2+}) is oxidized to ferric state (Fe^{3+}) and may hydrolyze to form red, insoluble hydrated ferric oxide. Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1,10-phenanthroline at pH (3.2-3.3). Three molecules of phenanthroline chelate each atom of ferrous iron to form orange red complex measurable at 510 nm.

Equipment:

1. Spectrophotometer, or filter photometer for use at 510 nm.
2. Nessler tubes.

Reagents:

1. Hydrochloric acid, (concentrated HCl).
2. Hydroxylamine solution. Dissolve 10 g hydroxylamine hydrochloride ($NH_2OH.HCl$) in 100 ml distilled water.
3. Ammonium acetate buffer solution ($NH_4C_2H_3O_2$). Dissolve 250 g ($NH_4C_2H_3O_2$) in 150 ml distilled water. Add 700 ml of concentrated (glacial) acetic acid. Good grade of ($NH_4C_2H_3O_2$) may contain significant amount of iron impurities. Prepare a new reference standard with each buffer preparation.
4. Phenanthroline solution. Dissolve 100 g (1,10-phenanthroline monohydrate) $C_{12}H_8N_2.H_2O$ in 100 ml distilled water by stirring and heating to 80 ºC, do not boil. Discard the solution, if it darkens. Heating is unnecessary if 2 drops concentrated HCl are added to the water.
5. Potassium permanganate, 0.02M (0.1N). Dissolve 0.316 g $KMNO_4$ in reagent water and dilute to 100 ml distilled water.
6. Stock iron solution. If ferrous ammonium sulfate is preferred, slowly add 20 ml of concentrated H_2SO_4 to 50 ml water and dissolve 1.404 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in the solution. Slowly add potassium permanganate until a faint pink color persists (approximately 50 ml of potassium permanganate is required), dilute to 1000 ml with water and mix (1.0 ml ≡ 200 µg Fe).
7. Standard iron solution. Pipet 50 ml of stock solution into 1000 ml volumetric flask and dilute to mark with water (1.0 ml ≡ 10 µg Fe).

Note:

Hydrochloric acid and ammonium acetate buffer solution are stable indefinitely if tightly stoppered.

- Hydroxylamine solution, phenanthroline and stock iron solution are stable for several months.
- The standard iron solutions are not stable, prepare daily as needed by diluting the stock iron solution.

Procedure:

1. Prepare a series of standard solution by accurately pipet of calculated volumes of standard iron solutions into flasks and dilute to 50 ml by adding measured volumes of water.
2. Take 50 ml of sample and add to both sample and standard, 2 ml of concentrated HCl and 1 ml ($NH_2OH.HCl$).
3. Add a few glass beads and heat to boiling to insure dissolution of all the iron, continue boiling until volume is reduced to 15 - 20 ml. If the sample is ashed, take up the residue in 2 ml concentrated HCl and 5 ml water.
4. Cool to room temperature and transfer to 100 ml volumetric flask or Nessler tube, add 10 ml ($NH_4C_2H_3O_2$) buffer solution and 4 ml phenanthroline solution, and dilute to mark with water. Mix thoroughly and allow standing for a minimum of 10 min for maximum color development.
5. Measure the developed color photometrically at 510 nm and compare with a calibration curve using a distilled water blank.

Calculation:

$$\text{Fe mg / l} = \frac{\mu\text{g Fe in 100 ml final volume}}{\text{ml sample}}$$

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 4500-D).

(III.3) Chromium (VI)

(Spectrophotometry - Diphenylcarbazide Method)

Principle:

This procedure measures only hexavalent chromium (Cr^{6+}). Therefore, to determine total chromium content (Cr^{3+} and Cr^{6+}), convert all chromium to the hexavalent state by a prior oxidation. The sample is digested with sulfuric-nitric acid mixture and oxidized with potassium permanganate. The hexavalent chromium is determined colorimetry by a reaction with diphenylcarbazide in an acid solution. A red-violet color is formed and the intensity of the color is sensitive at 540 nm.

Equipment:

1. Spectrophotometer, for use at 540 nm, with a light path of 1.0 cm or longer. Or a filter photometer, providing a light path of 1.0 cm or longer and equipped with a greenish-yellow filter having maximum transmittance near 540 nm.
2. Separatory funnels, 125-ml, Squibb form, with glass or Teflon stopcock and stopper.

Reagents:

Use redistilled water to prepare reagents.

1. Stock chromium solution: Dissolve 141.4 mg $K_2Cr_2O_7$ in water and dilute to 10 ml (1.00 ml ≡ 500 μg Cr).
2. Standard chromium solution: Dilute 1.00 ml stock chromium solution to 100 ml (1.00 ml ≡ 5.00 μg Cr).
3. Nitric acid, HNO_3, concentrated.
4. Sulfuric acid, H_2SO_4, concentrated.
5. Sulfuric acid, H_2SO_4, (1+1).
6. Sulfuric acid, H_2SO_4, 6 N.
7. Sulfuric acid, H_2SO_4, 0.2 N: Dilute 17 ml of 6 N H_2SO_4 to 500 ml with water.
8. Phosphoric acid, H_3PO_4, concentrated.
9. Methyl orange indicator solution.
10. Hydrogen peroxide, H_2O_2, 30%.
11. Redistilled water: Distilled water is redistilled in all-glass apparatus.
12. Aqueous ammonia solution, concentrated.
13. Potassium permanganate solution: Dissolve 4 g $KMnO_4$ in 100 ml water.
14. Sodium azide solution: Dissolve 0.5 g NaN_3 in 100 ml water.
15. Diphenylcarbazide solution: Dissolve 250 mg of 1,5-diphenylcarbazide in 50 ml acetone. Store in a brown bottle. Discard when the solution becomes discolored.
16. Chloroform, $CHCl_3$: Avoid or redistill material that comes in containers with metal or metal-lined caps.
17. Cupferron solution: Dissolve 5 g of the reagent $C_6H_5N(NO)ONH_4$ in 95 ml water.

Procedure:

1. Preparation of calibration curve: To compensate for possible slight losses of chromium during digestion or other analytical operations, treat chromium standards by the same procedure used with the test sample. Accordingly, pipet measured volumes of standard chromium solution (5 μg/ml) ranging from 2.00 to 20.0 ml, to give standards for 10 to 100 μg Cr, into 250-ml beakers or conical flasks. Depending on the used pretreatment method, proceed with subsequent treatment of standards as if they are test samples. Treat the standards with cupferron reagent, if this step is required for the test sample.
2. Color development for samples: Transfer a suitable portion of each colored solution to a 1.0-cm absorption cell, and measure absorbance at 540 nm. As reference, use distilled water. Correct absorbance readings of standards by subtracting absorbance reagent blank carried through the method.
3. Construction of the calibration curve by plotting corrected absorbance values against micrograms of chromium in 102 ml final volume.
4. Treatment of sample: If sample has been filtered and only chromium is measured, start analysis within 24 h of collection. If total dissolved chromium is to be measured and there are interfering amounts of molybdenum, vanadium, copper, or iron present, proceed as below. If interferences are not present, proceed as below. If the sample is unfiltered and total chromium is measured, digest the sample with a mixture of HNO_3 and H_2SO_4.
5. Removal of molybdenum, vanadium, iron, and copper with cupferron: Pipet a portion of digested sample containing 10 to 100 μg chromium into a 125 - ml separatory funnel. Dilute to about 40 ml with distilled water and chill in an ice bath. Add 5 ml ice cold cupferron solution, shake well, and let stand in ice bath for 1 min. Extract in separatory funnel with three successive 5-ml portions of $CHCl_3$; shake each portion thoroughly with aqueous solution, let layers separate, and withdraw and discard $CHCl_3$ extract. Transfer the extracted aqueous solution to a 125-ml conical flask. Wash separatory funnel with a small amount of distilled water and add wash water to flask. Boil for about 5 min to volatilize $CHCl_3$ and cool. Add 5 ml HNO_3 and 3 ml H_2SO_4. Boil samples to the appearance of SO_3 fumes. Cool slightly, carefully add 5 ml HNO_3, and again boil to fumes to complete decomposition of organic matter. Cool, wash sides of flask and boil once more to SO_3 fumes to eliminate all HNO_3. Cool and add 25 ml water.
6. Oxidation of trivalent chromium: Pipet a portion of digested sample with or without interferences removed, and containing 10 to 100 μg Cr, into a 125-ml conical flask. Add several drops of methyl orange indicator, and then add concentrated aqueous ammonia until solution just begins to turn yellow. Add (1+1) H_2SO_4 dropwise until it is acidic, plus 1 ml (20 drops) in excess. Adjust volume to about 40 ml, add a boiling chip, and heat to boiling. Add 2 drops $KMnO_4$ solution to give a dark red color. If fading occurs, add $KMnO_4$ dropwise to maintain an excess of about 2 drops. Boil for 2

min, add 1 ml of NaN_3 solution and continue boiling gently. If red color doesn't fade completely after boiling for approximately 30 s, add another 1 ml NaN_3 solution. Continue boiling for 1 minute after color has faded completely then cool. Add 0.25 ml (5 drops) H_3PO_4.

7. Color development and measurement: Use 0.2 N H_2SO_4 and a pH meter to adjust solution to pH 1.0±0.3. Transfer the solution to a 100 ml volumetric flask. Dilute to 100 ml, mix and add 2.0 ml diphenylcarbazide solution. Mix, and let stand 5 to 10 min for full color development. Transfer an appropriate portion of the solution to a 1.0-cm absorption cell and measure absorbance at 540 nm. Use distilled water as a reference. Correct the absorbance reading of the sample by subtracting absorbance of a blank carried through the method. From the corrected absorbance determine micrograms chromium present by reference to the calibration curve.
8. Note*:* If the test sample solution is turbid after dilution to 100 ml, take an absorbance reading before adding diphenylcarbazide reagent and correct absorbance reading of final colored solution by subtracting the absorbance measured previously.

Calculation:

$$\text{Chromium (VI), (mg/l)} = \frac{\mu\text{g Cr in 102 ml final volume}}{A \times B} \times 100$$

where:

A = volume (ml) of original sample.

B = volume (ml) from 100 ml digested sample.

Precautions:

1. The reaction with diphenylcarbazide is nearly specific for Cr^{6+}.
2. Hexavalent molybdenum and mercury salts develop color with the reagent but the intensities are much lower than that for chromium at the specified pH.
3. Vanadium interferes strongly but concentrations up to 10 times that of chromium will not cause trouble.
4. Potential interference from permanganate is eliminated by prior reduction with azide.
5. Iron in concentrations greater than 1 mg/l may produce a yellow color but the ferric ion (Fe^{3+}) color is not strong and no difficulty is encountered normally if the absorption is measured photometrically at the appropriate wavelength.
6. Interfering amounts of molybdenum, vanadium, iron, and copper are removed by extraction of the cupferrone derivatives of these metals into chloroform ($CHCl_3$). This step is not used unless necessary.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association, Washington 2017 [Method (3500-Cr (D)].

(III.4) Boron

(Spectrophotometry - Curcumin Method)

Principle:

When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red colored product called rosocyanine is formed. Rosocyanine is extracted in a suitable solvent and the intensity of the red color developed is measured at 540 nm and compared with standards visually or photometrically.

Equipment:

1. Spectrophotometer, for use at 540 nm, with a minimum light path of 1.0 cm or filter photometer, equipped with a green filter (maximum transmittance near 540 nm), and a minimum light path of 1.0 cm.
2. Evaporating dishes, 100 - 150 ml capacity, of high silica glass, platinum, or other suitable materials.
3. Water bath, set at 55±2°C.
4. Glass stoppered volumetric flasks, 25 and 50 ml capacity.
5. Ion exchange column, 50 cm long and 1.3 cm diameter.

Reagents:

1. Store all reagents in polyethylene or boron free containers.
2. Stock boron solution: Dissolve 571.6 mg anhydrous boric acid, H_3BO_3, in distilled water and dilute to 1000 ml (1.00 ml ≡ 1.00 μg B).
3. Standard boron solution: Dilute 10.00 ml stock boron solution to 1 liter with distilled water (1.00 ml ≡ 0.01 μg B).
4. Curcumin reagent: Dissolve 40 mg finely ground curcumin and 5.0 g oxalic acid in 80 ml 95% ethyl alcohol. Add 4.2 ml concentrated HCl, make up to 100 ml with ethyl alcohol in a 100 ml volumetric flask, and filter if reagent is turbid (isopropyl alcohol, 95% may be used in place of ethyl alcohol). This reagent is stable for several days, if stored in a refrigerator.
5. Ethyl or isopropyl alcohol, 95 %.
6. Reagents for removal of high hardness and cation interference: Strongly acidic cation exchange resin.
7. Hydrochloric acid, HCl, (1+5).

Procedure:

1. Preparation of calibration curve: Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 μg boron into evaporating dishes of the same type, shape, and size. Add distilled water to each standard to bring total volume to 1.0 ml. Add 4.0 ml curcumin reagent to each and swirl gently to mix contents thoroughly. Heat the dishes on a water bath at 55±2 °C for

80 min., which is usually sufficient for complete dissolution of the red colored product. Wash the contents of dish into a 25 ml volumetric flask, using 95% ethyl alcohol. Make up to mark with 95 % ethyl alcohol and mix thoroughly. Read absorbance of standards and samples at 540 nm after setting reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 µg boron. Make photometric readings within 1 h of drying samples.

2. Sample treatment: For waters containing 0.10 to 1.00 mg B/l, use 1.00 ml sample. For waters containing more than 1.00 mg B/l, make an appropriate dilution with boron-free distilled water, so that a 1.00 ml portion contains approximately 0.50 µg boron. Pipet 1.00 ml sample or dilution into an evaporating dish. Unless the calibration curve is being determined at the same time, prepare blank and standard containing 0.50 µg boron and run in conjunction with the sample. Add 4.0 ml curcumin reagent and complete as above. If the final solution is turbid, filter through filter paper before reading absorbance. Calculate boron content from calibration curve.
3. Removal of high hardness and cation interference: Prepare an ion-exchange column of approximately 20 cm x 1.3 cm diameter. Charge column with a strongly acidic cation exchange resin. Backwash column with distilled water to remove entrained air bubbles. Keep the resin covered with liquid at all times. Pass 50 ml (1+5) HCl through column at a rate of 0.2 ml acid/ml. Wash column with distilled water till free from acid. Pipet 25 ml sample, or a smaller sample volume of known high boron content diluted to 25 ml, onto the resin column. Adjust the rate of flow to about 2 drops/s and collect effluent in a 50 ml volumetric flask. Wash column with small portions of distilled water until flask is filled to mark. Mix and transfer 2.00 ml into evaporating dish. Add 4.0 ml curcumin reagent and complete the analysis as described above.

Calculation:

Use the following equation to calculate boron concentration from absorbance readings:

$$\text{Boron, (mg /l)} = \frac{A_2 \times C}{A_1 \times S}$$

where:

A_1 = absorbance of standard.

A_2 = absorbance of sample.

C = µg B in standard taken.

S = volume (ml) of sample.

Precautions:

1. Closely control such variables as volumes and concentrations of reagents, as well as time and temperature of drying.
2. Use evaporating dishes identical in shape, size, and composition to insure equal evaporation time because increasing the time increases intensity of the resulting color.

3. Nitrate-N concentrations above 20 mg/l interfere. Significantly high results are possible when the total calcium and magnesium hardness exceed 100 mg/l as calcium carbonate ($CaCO_3$).
4. Moderate hardness levels may cause a considerable percentage of error in the low boron range. This interference is due to the insolubility of the hardness salts in 95% ethanol and consequent turbidity in the final solution.
5. Filter the final solution or pass the original sample rough a column of strongly acidic cation-exchange resin in the hydrogen form to remove interfering cations. The latter procedure permits application of the method to samples of high hardness or solids content. Phosphate does not interfere.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association, Washington 2017 [Method (3500-Cr (D)].

(III.5) Metal Constituents

(Sample Preparation Before Spectral Atomic Analysis)

Principle:

Methods used for preparing surface and ground water samples for analysis by atomic absorption spectroscopy (AAS) or by inductively coupled plasma spectroscopy (ICP) are commonly involve a prior open or microwave acid digestion using (HNO_3), (HNO_3+HCl), (HNO_3+$HClO_4$), or (HNO_3+$HClO_4$+HF). Dry ashing may also be used followed by acid dissolution. These treatments convert the metal contents into ionic forms. Elements listed in Table (III.1) are determined using these techniques.

Table (III.1) Analysis of some metals using AAS or ICP

Aluminum	Beryllium	Cobalt	Manganese	Selenium	Vanadium
Antimony	Cadmium	Copper	Molybdenum	Silver	Zinc
Arsenic	Calcium	Iron	Nickel	Sodium	Lead
Barium	Chromium	Magnesium	Potassium	Thallium	

Introduction:

1. Total recoverable metals. The entire sample is acidified at the time of collection with nitric acid. At the time of analysis, the sample is heated with acid and substantially reduced in volume. The digestate after filtration and dilution to volume, is ready for analysis.
2. Dissolved metals. The sample is filtered through a 0.45-µm filter at the time of collection and the sample is acidified at the time of collection with nitric acid. Samples tested for dissolved metals do not need to be digested, as long as the acid concentrations have been adjusted to the same concentration as in the standards.
3. Collection and preservation. For the determination of dissolved metals, the sample must be filtered through 0.45 µm pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of the filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH < 2.
4. For total recoverable metals in aqueous samples, sample filteration is not required, but acidify with (1+1) nitric acid to pH < 2 (normally, 3 ml of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection.
5. For direct analysis of total recoverable metals in drinking water samples containing turbidity < 1 NTU, treat an unfiltered acid preserved sample aliquot.
6. For determination of total recoverable metals in aqueous samples (other than drinking water with > 1 NTU turbidity), digestion with acid is required.

Equipment:

1. Griffin beakers of assorted sizes or equivalent.
2. Watch glasses or equivalent.
3. Qualitative filter paper and filter funnels.
4. Graduated cylinder or equivalent.
5. Electric hot plate or equivalent-adjustable and capable of maintaining a temperature of 90 - 95 ^{o}C.

Reagents:

1. Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee of Analytical Reagents of the American Chemical Society, where such specifications are available.
2. Other grades may be used provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without affecting the accuracy of the determination.
3. Reagent water. Reagent water shall be interference free. All references to water in the method refer to reagent water unless otherwise specified.
4. Nitric acid (concentrated), HNO_3.
5. Hydrochloric acid (concentrated), HCl.
6. The acids (HNO_3 and HCl) should be analyzed to determine the level of impurities. If method blank is < MDL, then the acid can be used.

Procedure:

1. Transfer a 100 ml aliquot of a well-mixed sample to a beaker.
2. Add 2 ml of concentrated HNO_3 and 5 ml of concentrated HCl. The sample is covered with a ribbed watch glass or other suitable covers and heated on a steam bath, hot plate or other heating source at 90 to 95 ^{o}C until the volume has been reduced to 15 - 20 ml.
3. Do not boil the test solution because antimony, if present, will be easily lost by volatilization from hydrochloric acid media.
4. Remove the beaker and allow cooling. Wash down the beaker walls and watch glass with water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.
5. Filtration step should be done only if there is a concern about the presence of insoluble materials; this additional step is liable to cause sample contamination unless the filter and filtering apparatus are thoroughly cleaned with dilute HNO_3.
6. Adjust the final volume to 100 ml with reagent water.

Interferences:

1. The digestion procedure may not be sufficient to destroy some metal complexes.
2. If silver is present, precipitation of AgCl will cause a lowering of the silver concentration and therefore an inaccurate analysis.

3. When determined arsenic and selenium by ICP-MS, do not used HCl in the digestion method but use HNO_3 only to avoid polyatomic molecular interference by Cl and formation of argon chloride.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association, Washington 2017 [Method (3030(F)].

(III.6) Metal Constituents

(Flame Atomic Absorption Spectrometry)

Principle:

Methods used for determining metals by atomic absorption spectrometry vary from one element to another depending on the nature and concentration level of the element to be measured. In these methods the test sample is aspirated into a flame and atomized. A light beam from a hollow cathode lamp is directed through the flame and onto the detector to measure the amount of light absorbed by the atomized element in the flame. Direct air-acetylene flame method is used for determining sodium, magnesium, lithium, potassium, , iron, zinc, manganese, antimony, cadmium, chromium, cobalt, copper, lead, nickel, silver, palladium, platinum, rhodium, ruthenium, strontium, gold, cesium, thallium, iridium, bismuth, and tin. Direct nitrous oxide-acetylene flame method is used for determining aluminum, barium, beryllium, calcium, molybdenum, titanium, osmium, rhenium, silicon, thorium and vanadium. Extraction of the metal with methyl isobutyl keton (MIBK) may be used to increase the sensitivity of the method.

Table (III.2) Metal concentration ranges with direct aspiration atomic absorption

Element	Wave-length, nm	Flame gases	Instrument detection level, mg/l	Sensitivity, mg/l	Optimum concentration range, mg/l
Ag	328.1	A–Ac	0.01	0.06	0.1–4
Al	309.3	N–Ac	0.1	1	5–100
Au	242.8	A–Ac	0.01	0.25	0.5–20
Ba	553.6	N–Ac	0.03	0.4	1–20
Be	234.9	N–Ac	0.005	0.03	0.05–2
Bi	223.1	A–Ac	0.06	0.4	1–50
Ca	422.7	A–Ac	0.003	0.08	0.2–20
Cd	228.8	A–Ac	0.002	0.025	0.05–2
Co	240.7	A–Ac	0.03	0.2	0.5–10
Cr	357.9	A–Ac	0.02	0.1	0.2–10
Cs	852.1	A–Ac	0.02	0.3	0.5–10
Cu	324.7	A–Ac	0.01	0.1	0.2–10
Fe	248.3	A–Ac	0.02	0.12	0.3–15
Ir	264.0	A–Ac	0.6	8	-
K	766.5	A–Ac	0.005	0.04	0.1–2
Li	670.8	A–Ac	0.002	0.04	0.1–2
Mg	285.2	A–Ac	0.0005	0.007	0.02–2
Mn	279.5	A–Ac	0.01	0.05	0.1–10
Mo	313.3	N–Ac	0.1	0.5	1–20
Na	589.0	A–Ac	0.002	0.015	0.03–1
Ni	232.0	A–Ac	0.02	0.15	0.3–10

Os	290.9	N–Ac	0.08	1	—
Pb	283.3	A–Ac	0.05	0.5	1–20
Pt	265.9	A–Ac	0.1	2	5–75
Rh	343.5	A–Ac	0.5	0.3	—
Ru	349.9	A–Ac	0.07	0.5	—
Sb	217.6	A–Ac	0.07	0.5	1–40
Si	251.6	N–Ac	0.3	2	5-150
Sn	224.6	A–Ac	0.8	4	10-200
Sr	460.7	A–Ac	0.03	0.15	0.3-5
Ti	365.3	N–Ac	0.3	2	5-100
V	318.4	N–Ac	0.2	1.5	2-100
Zn	213.9	A–Ac	0.005	0.02	0.05-2

Equipment:

1. Atomic absorption spectrometer. It consists of a light source emitting the line spectrum of an element (hollow-cathode lamp or electrodeless discharge lamp), a device for vaporizing the sample (usually a flame or graphite furnace), a means of isolating an absorption line (monochromator or filter and adjustable slit), and a photo-electric detector with its associated electronic amplifying and measuring equipment.
2. Lamps. Use either a hollow-cathode lamp or an electrodeless discharge lamp (EDL).
3. Pressure-reducing valves.
4. Vent for direct nitrous oxide-acetylene flame system.
5. Nitrous oxide burner head as suggested in manufacturer's manual.
6. T-junction valve.

Reagents:

1. Air. Cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The source may be a compressor or commercially bottled gas.
2. Acetylene. Standard commercial grade. Acetylene, which always is present in acetylene cylinders, can be prevented from entering and damaging the burner head by replacing a cylinder when its pressure has fallen to 689 kPa (100 psi) acetylene. Caution: Acetylene gas is an explosive hazard in the laboratory. Follow instrument manufacturer's directions in plumbing and using this gas.
3. Metal-free water. Use metal-free water for preparing all reagents and calibration standards and as dilution water.
4. Hydrochloric acid: HCl, 1 %, 10 %, 20 %, (1 + 5), (1 + 1), all (v/v), and concentrated.
5. Nitric acid: HNO_3, 2 % (v/v), (1 + 1), and concentrated.
6. Aqua regia: Add 3 volumes of concentrated HCl to 1 volume of concentrated HNO_3 and mix.
7. Standard metal solutions. Prepare a series of standard metal solutions in the optimum concentration range by appropriate dilution of the stock metal solutions with water

containing 5 % HNO_3. Stock standard solutions are available from a number of commercial suppliers.

8. Nitrous oxide, commercially available cylinders.

Prcedure:

A. Sample preparation:

Sample preparation depends on the metal form being measured. If dissolved metals or total acid-extractable metals are to be determined, see the section above for sample preparation. For all samples, make certain that the concentrations of acid and matrix modifiers are the same in both samples and standards.

B. Instrument operation with direct air-acetylene flame method:

See manufacturer's operating manual. In general, proceed according to the following:

1. Install a hollow-cathode lamp for the desired metal in the instrument and roughly set the wavelength dial according to Table (III. 2).
2. Set slit width according to manufacturer's suggested setting for the element being measured.
3. Turn on instrument, apply to the hollow-cathode lamp the current suggested by the manufacturer, and let instrument warm up until energy source stabilizes, generally about 10 to 20 min.
4. Readjust current as necessary after warm up.
5. Optimize wave-length by adjusting wavelength dial until optimum energy gain is obtained. Align lamp in accordance with manufacturer's instructions.
6. Install suitable burner head and adjust burner head position. Turn on air and adjust flow rate to that specified by manufacturer to give maximum sensitivity for the metal being measured. Turn on acetylene, adjust flow rate to value specified, and ignite flame.
7. Let flame stabilize for a few minutes, aspirate a blank consisting of deionized water containing the same concentration of acid in the standards and samples and Zero the instrument.
8. Aspirate a standard solution and adjust aspiration rate of the nebulizer to obtain maximum sensitivity. Adjust burner both vertically and horizontally to obtain maximum response.
9. Aspirate the blank again, re-Zero the instrument and aspirate a standard near the middle of the linear concentration range. Record absorbance of this standard when freshly prepared and with a new hollow-cathode lamp.
10. Refer to these data on subsequent determinations of the same element to check consistency of instrument setup and aging of hollow-cathode lamp and standards.
11. The instrument now is ready to operate. When analyses are finished, extinguish flame by turning off first acetylene and then air.

C. Instrument operation with direct nitrous oxide-acetylene flame method:

1. Adjust wave-length; install a nitrous oxide burner head and turn on acetylene (without igniting flame) and adjust flow rate to value specified by manufacturer for a nitrous oxide-acetylene flame. Turn off acetylene.
2. With both air and nitrous oxide supplies turned on, set T-junction valve to nitrous oxide and adjust flow rate according to manufacturer's specifications.
3. Turn switching valve to the air position, verify that flow rate is the same and turn acetylene on and ignite to a bright yellow flame.
4. Turn switching valve to nitrous oxide where the flame should have a red cone above the burner. If it does not, adjust fuel flow to obtain red cone.
5. After nitrous oxide flame has been ignited, let burner come to thermal equilibrium before beginning analysis. Aspirate a blank consisting of deionized water containing 1.5 ml concentrated HNO_3 /l and check aspiration rate.
6. Adjust if necessary to a rate between 3 and 5 ml/min. Zero the instrument. Aspirate a standard of the desired metal with a concentration near the midpoint of the optimum concentration range and adjust burner (both horizontally and vertically) in the light path to obtain maximum response.
7. Aspirate blank again and re-Zero the instrument. The instrument now is ready to run standards samples.
8. To extinguish flame, turn switching valve from nitrous oxide to air and turn off acetylene. This procedure eliminates the danger of flashback that may occur on direct ignition or shutdown of nitrous oxide and acetylene.

D. Standardization:

1. Select at least three concentrations of each standard metal solution to bracket the expected metal concentration of the sample. Aspirate blank and zero the instrument. Then aspirate each standard in turn into flame.
2. Rinse the nebulizer by aspirating water containing 1.5 ml of concentrated HNO_3. Aspirate blank, zero instrument, aspirate sample and determine its concentration.

Calculation:

Calculate the concentration of each metal ion, in micrograms per liter for trace elements, and in milligrams per liter for more common metals, if the sample has been diluted, multiply by the appropriate dilution factor.

Interferences:

1. Chemical interference. Many metals can be determined by direct aspiration of sample into an air-acetylene flame. Some types of interferences are termed "chemical" and results from the lack of absorption by atoms due to formation of molecular species (metal oxide, carbide and nitride) in the flame. This can occur when the flame is not hot enough to dissociate the molecules species or when the dissociated atom is oxidized immediately to a compound that will not dissociate further at the flame

temperature. Such interferences may be reduced or eliminated by adding specific elements or compounds to the sample solution. For example, the interference of phosphate in the magnesium determination can be overcome by adding lanthanum. The nitrous oxide-acetylene flame also can be useful in minimizing certain types of chemical interferences encountered in the air-acetylene flame.

2. Background correction. Molecular absorption and light scattering caused by solid particles in the flame can cause erroneously high absorption values resulting in positive errors. When such phenomena occur, use background correction to obtain accurate values. Use any one of three types of background correction: continuum-source, Zeeman, or Smith-Hieftje correction.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association, Washington 2017 [Method (3111(B, D)].

(III.7) Metal Constituents

(Electrothermal (Flameless) Atomic Absorption Spectrometry)

Principle:

It is the same principle as direct flame atomization but an electrically heated atomizer or graphite furnace replaces the standard burner head. A discrete sample volume is dispensed into the graphite sample tube (or cup).Typically, determinations are made by heating the sample in three or more stages. The resultant ground-state atomic vapor absorbs monochromatic radiation from the source. A photoelectric detector measures the intensity of transmitted radiation. The inverse of the transmittance is related logarithmically to the absorbance, which is directly proportional to the number density of vaporized ground-state atoms over a limited concentration range. This technique is used for determination of micro and trace quantities of most metallic elements (1 µg/l) with sensitivities and detection limits from 20 - 1000 times better than the flame technique. The accuracy of the method is less than that obtained by the flame technique and matrix modifiers are used. Metals such as aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, and tin are satisfactorily measured by this technique. Matrix modifiers are used to improve the accuracy of the method.

Equipment:

1. Atomic absorption spectrometer as described in the flame method.
2. Graphite furnace.

Reagents:

1. Metal-free water, use metal-free water for preparing all reagents and calibration standards and as dilution water.
2. Hydrochloric acid, HCl, 1 %, 10 %, 20 %, (1+5), (1+1), all (v/v), and concentrated.
3. Nitric acid, HNO_3, 2 % (v/v), (1+1) and concentrated.
4. Aqua regia, add 3 volumes of concentrated HCl to 1 volume of concentrated HNO_3.
5. Standard metal solutions. Prepare a series of standard metal solutions in the optimum concentration range by appropriate dilution of the stock metal solutions with water containing 5 % concentrated HNO_3/l. Stock standard solutions are available from a number of commercial suppliers.

Procedure:

A. Sample pretreatment:

1. Rinse all glassware with (1:1) HNO_3 and water and carry out digestion procedures in a clean, dust-free laboratory area to avoid sample contamination. For digestion of trace

aluminum, use polypropylene or Teflon utensils to avoid leachable aluminum from glassware.
2. Samples with dissolved metals and turbidity > 1 NTU should be digested.
3. Metals such as (Al, Sb, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Ag, and Sn) are not recovered unless HCl is used in the digestion.
4. For total recoverable As and Se, transfer 100 ml of shaken sample, 1 ml concentrated HNO_3, and 2 ml 30 % H_2O_2 to a clean, acid-washed 250-ml beaker. Heat on a hot plate without allowing solution to boil until volume has been reduced to about 50 ml. Remove from hot plate and let cool to room temperature and dilute to volume in a 100-ml volumetric flask with water.

*B. Instrument operation***:**

1. Mount and align furnace device according to manufacturer's instructions.
2. Turn on instrument and data collection system.
3. Select appropriate light source and adjust to recommend electrical setting.
4. Select proper wavelength and set all conditions according to manufacturer's instructions, including background correction.

C. Standardization:

Prepare a series of standard metal solutions in the optimum concentration range by appropriate dilution of the stock metal solutions with water containing 5 % concentrated HNO_3/l. Stock standard solutions are available from a number of commercial suppliers.

D. Analysis of samples:

Analyze all samples at least in duplicate or until reproducible results are obtained. A variation of 10 % is considered acceptable reproducibility Average replicate values

Precautions:

Use low levels of palladium (50 to 250 mg/l) for normal samples and higher levels for complex samples. Addition of excess palladium modifier may widen atomization peaks; in such cases peak area measurements may provide higher quality results. The recommended mode of modifier use is through co-addition to the furnace of about 10 µl of the palladium (or other) modifier solution. Palladium may not be the best modifier in all cases and cannot be recommended unconditionally. Test samples require a modifier with palladium; test other modifiers only if palladium is unsuccessful or to minimize modifier cost.

Interferences:

1. Electrothermal atomization determinations may be subject to significant interferences from molecular absorption as well as chemical and matrix effects.
2. Molecular absorption may occur when components of the sample matrix volatilize during atomization, resulting in broadband absorption.
3. Several background correction techniques are available commercially to compensate for this interference.

4. Nonetheless, the use of matrix modifiers is encouraged. Some specific chemical modifiers and approximate concentrations are listed inTable (III. 3) addition of a chemical modifier directly to the sample before analysis is restricted to inexpensive additives (e.g., phosphoric acid).
5. Use of palladium salts for matrix modification normally requires methods of co-addition, in which sample and modifier are added consecutively to the furnace either manually or, preferably, with an automatic sampler. Palladium salts (nitrate is preferred, chloride is acceptable) are listed in Table (III.3) as a modifier for many metals.
6. The palladium solution (50 to 2000 mg/l) generally includes citric or ascorbic acid, which aids reduction of palladium in the furnace. Citric acid levels of 1 to 2 % are typical. Use of hydrogen (5 %) in the purge gas (available commercially as a mixture) also reduces palladium, eliminating need for organic reducing acids.

Table (III. 3) Matrix modifiers used with flameless atomic absorption spectrometry

	Modifier	Analyses for which modifier may be useful
1	500 mg Pd/l +1000 mg $Mg(NO_3)_2$/l	Ag, As, Au, Bi, Cu, Ge, Mn, Hg, In, Sb, Se, Sn, Te, T
2	500–2000 mg Pd/l reducing agent	Ag, As, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb
3	5000 mg $Mg(NO_3)_2$/l	Be, Co, Cr, Fe, Mn, V
4	100–500 mg Pd/l	As, Ga, Ge, Sn
5	50 mg Ni/l	As, Se, Sb
6	2% PO_4 1000 mg Mg $(NO_3)_2$ /l	Cd, Pb

Calculation:

Calculate concentration of each metal ion, in micrograms per liter for trace elements, if the sample has been diluted, multiply by the appropriate dilution factor.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method 3113A.

(III.8) Mercury

(Cold - Vapor Atomic Absorption Spectrometry)

Principle:

This method is used for determination of mercury ions. The mercury salts are treated with a reducing agent ($NaBH_4$, $LiAlH_4$, Sn^{2+}, hydroxylamine) to generate free mercury atoms at room temperature. The atoms are swept to a closed glass cell and allowed to interact with the radiation from a mercury lamp. The method neither involves the use of flame or furnace atomization device nor the use of hollow cathode lamp.

Equipment:

1. Atomic absorption spectrometer and associated equipment in addition to accessories specifically designed for measuring mercury *via* the cold vapor technique.
2. Absorption cell, a glass or plastic tube approximately 2.5 cm in diameter
3. Cell support to strap the cell to the flat nitrous-oxide burner head or other suitable support and align in light beam to give maximum transmittance.
4. Air pumps, any peristaltic pump with electronic speed control capable of delivering an air flow of 2 liter/min.
5. Regulated compressed air system or air cylinder also is satisfactory.
6. Flowmeter, capable of measuring an air flow of 2 liter/min.
7. Aeration tubing, a straight glass frit having a coarse porosity for use in reaction flask.
8. Reaction flask, 250-ml Erlenmeyer flask or a BOD bottle, fitted with a rubber stopper to hold aeration tube.
9. Drying tube, 150-mm long and 18-mm-diam, containing 20 g $Mg(ClO_4)_2$. A 60-W light bulb with a suitable shade may be substituted to prevent condensation of moisture inside the absorption cell.
10. Connecting tubing, glass tubing to pass mercury vapor from reaction flask to absorption cell and to interconnect all other components.

Reagents:

1. Metal-free water.
2. Stock mercury solution.
3. Standard mercury solutions.
4. Nitric acid, HNO_3, concentrated.
5. Potassium permanganate solution. Dissolve 50 g $KMnO_4$ in water and dilute to 1 liter.
6. Potassium persulfate solution. Dissolve 50 g $K_2S_2O_8$ in water and dilute to 1 liter.
7. Sodium chloride-hydroxylamine sulfate solution: Dissolve 120 g NaCl and 120 g $(NH_2OH)_2H_2SO_4$ in water and dilute to1 liter.
8. A 10 % hydroxylamine hydrochloride solution may be substituted for the hydroxylamine sulfate.

9. Stannous ion (Sn^{2+}) solution. Dissolve 10 g $SnCl_2$ in water containing 20 ml concentrated HCl and dilute to 100 ml.
10. Dissolve 11 g $SnSO_4$ in water containing 7 ml concentrated H_2SO_4 and dilute to 100 ml.
11. Sulfuric acid, H_2SO_4, concentrated.

Procedure:

1. Transfer 100 ml of test sample or portion diluted to 100 ml containing not more than 5.0 g Hg/l to a reaction flask.
2. Seawaters, brines, and effluents high in chlorides require as much as another 25 ml $KMnO_4$ solution.
3. During oxidation step, chlorides are converted to free chlorine, which absorbs at 253 nm. Remove all free chlorine before the Hg is reduced and swept into the cell by using an excess (25 ml) of hydroxylamine reagent.
4. Remove free chlorine by passing gently air or nitrogen gas through the sample after adding hydroxylamine reducing solution.
5. Use a separate tube and frit to avoid carryover of residual stannous chloride, which could cause reduction and loss of mercury.

Calculation:

Determine peak height of sample from recorder chart and read mercury value from standard curve prepared.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method 3112 (B).

(III.9) Metal Constituents

(Inductively Coupled Plasma, ICP- OES)

Principle:

ICP-OES (inductively coupled plasma/optical emission spectrometry) is mainly used for samples with high total dissolved solids (TDS) or suspended solids and is, therefore, more robust for analyzing ground water, wastewater, soil, and solid waste. It can be used for drinking water analysis as well. A sample aerosol is generated in an appropriate nebulizer and spray chamber and is carried into the plasma through an injector tube located within the torch. The sample aerosol is injected directly into the ICP, subjecting the constituent atoms to temperatures of about 6000 to 8000°K offering efficient excitation and resulted in low detection limits for many elements. This method has been demonstrated to be suitable for metals in Table (III. 4).

Table (III. 4) Suggested suitable wavelength (nm) for ICP measurements of some metals

Element	Suggested wavelength, nm
Aluminum	308.22
Antimony	206.83
Arsenic	193.70
Barium	455.40
Beryllium	313.04
Boron	249.77
Cadmium	226.50
Calcium	317.93
Chromium	267.72
Cobalt	228.62
Copper	324.75
Iron	259.94
Lead	220.35
Lithium	670.78
Magnesium	279.08
Manganese	257.61
Molybdenum	202.03
Nickel	231.60
Potassium	766.49
Selenium	196.03
Silicon	251.611
Silver	328.07
Sodium	589.00
Strontium	407.77
Thallium	190.86
Vanadium	292.40
Zinc	213.86

Equipment:

1. Inductively coupled plasma system equipped with an ICP source, mass flow controllers for regulating ICP gas flows, a peristaltic pump for introducing samples, and a computerized data acquisition and instrument control system. An x-y auto-sampler may also be used with appropriate control software.
2. Spectrometer for either simultaneous (polychromatic) or sequential (monochromatic) type with air-path, inert gas purged, or vacuum optics.
3. Laboratory ware, precleaned plastic laboratory ware for standard and sample preparation. Teflon is preferred for standard preparation and sample digestion.
4. Micro pipits, different sizes of 10 to 100 µl, 100 to 1000 µl, and 1 to 10 ml.
5. Analytical balance, accurate to 0.001 mg.
6. Sample-preparation apparatus such as hot plates, microwave digesters, and heated sand baths.
7. Clean hood.

Reagents:

1. Acid**s.** Hydrochloric acid, HCl, concentrated and (1:1) solution. Nitric acid, HNO_3, concentrated. Nitric acid, HNO_3, (1:1).
2. Reagent water. Metal free water is used for preparing blanks, standards, and samples.
3. Stock standard solutions. Preferably, high-purity.
4. Stock solutions. These are prepared and diluted to the required concentrations either for single- or multi-elements.
5. Calibration standards. A five standard calibration is recommended; from 0 to 10 mg/l. prepare all calibration standards and blanks in a matrix of 2 % nitric acid.
6. Calibration blank. Dilute 2 ml of (1:1) HNO_3 and 10 ml (1:1) HCl to 100 ml with water. Prepare a sufficient quantity to be used to flush the system between standards and samples.
7. Method blank. Prepare method blank to contain the same acid types and concentrations as the sample solutions.
8. Instrument check standard. It is prepared by combining compatible elements at a concentration of 2 mg/l.
9. Instrument quality control sample**.** A certified aqueous reference standard is obtained from an outside source and prepared according to instructions provided by the supplier. Use the same acid matrix as the calibration standards.
10. Argon. A high quality grade.

Procedures:

1. Sample preparation**.** See method preparation described above for general guidance and additional specific requirements on sampling and recommended sample digestion technique for all analytes.
2. Instrument operating conditions. Follow up manufacturer's standard operating procedures for initialization, calibration, gas flow optimization, and other instrument operating conditions.

3. Instrument calibration. Calibrate instrument according to manufacturer's recommended procedure using calibration standards and blank. Aspirate each standard or blank for a minimum of 15 s after reaching the plasma before beginning signal integration. Rinse with calibration blank or similar solution for at least 60 s between each standard to eliminate any carryover from the previous standard. Use average intensity of multiple integrations of standards or samples to reduce random error.
4. Sample analysis. Begin each sample run with an analysis of the calibration blank, and then analyze the method blank. This permits a check of the sample preparation reagents and procedures for contamination. Analyze samples, alternatingly with the analyses of calibration blank. Rinse for at least 60 s with dilute acid between samples and blanks.
5. After introducing each sample or blank let system equilibrate before starting signal integration. Examine each analysis of the calibration blank to verify that no carry-over memory effect has occurred. If carry-over is observed, repeat rinsing until proper blank values are obtained. Make appropriate dilutions and acidifications of the sample to determine concentrations beyond the linear calibration range.

Calculation and correction:

1. Blank correction. Subtract result of an adjacent calibration blank from each sample result to make a baseline drift correction.
2. Dilution correction**.** If the sample was diluted or concentrated during preparation, multiply results by the dilution factor (DF) and calculated as follows:

$$DF = \frac{\text{Final weight or volume}}{\text{Initial weight or volume}}$$

3. Correction for spectral interference. Correct for spectral interference by using computer software supplied by the instrument manufacturer or by using the manual method based on interference correction factors.

Interferences:

Interferences may be categorized as follows:

1. Spectral intreferences. Light emission from spectral sources other than the element of interest may contribute to apparent net signal intensity. Sources of spectral interference include direct spectral line overlaps, broadened wings of intense spectral lines, ion-atom recombination continuum emission, molecular band emission, and stray (scattered) light from the emission of elements at high concentrations. Avoid line overlaps by selecting alternate analytical wavelengths. Avoid or minimize other spectral interference by judicious choice of background correction positions.
2. Non-spectral interferences. These are:

- Physical interferences. These are due to differences in viscosity, surface tension, and dissolved solids between samples and calibration standards. To minimize these effects,

the test samples should not contain more than 0.5 % of dissolved solids. Dilute water and wastewater samples with higher dissolved solids levels before analyzing them.

- Chemical interferences. These are caused due to molecular compound formation, ionization effects, and thermo chemical effects associated with sample vaporization and atomization in the plasma. Normally, these effects are not pronounced and can be minimized by careful selection of operating conditions (incident power, plasma observation position, etc.). Chemical interferences are highly dependent on sample matrix and element of interest. As with physical interferences, compensate by using matrix matched standards or by using standard addition.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 3120 (B).

(III.10) Metal Constituents

(Inductively Coupled Plasma - Mass Spectrometry, ICP-MS)

Principle:

In this method, the sample material is introduced to an argon-based, high-temperature radio-frequency plasma, usually *via* pneumatic nebulization. As energy transfers from the plasma to the sample stream, the target element dissolves, atomizes, and ionizes. The resulting ions are extracted from the plasma through a differential vacuum interface and separated based on their mass-to-charge (m/z) ratio by a mass spectrometer. Typically, either a quadrupole (with or without CCT or DRC) or magnetic sector (high resolution) mass spectrometer is used. An electron multiplier detector counts the separated ions, and a computer-based data-management system processes the resulting information. The method is suitable for determining aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium, silver, strontium, thallium, uranium, vanadium, and zinc metals. It is also acceptable for other elemental analyses as long as the same quality assurance practices are followed, with documented acceptance limits as sodium, calcium, boron, and iron (Table III. 5).

Table (III. 5) Recommended mass numbers

No	Element	Recommended mass No.
1	Be	9
2	Al	27
3	V	51
4	Cr	52
5	Cr	53
6	Mn	55
7	Co	59
8	Ni	60
9	Ni	62
10	Cu	63
11	Cu	65
12	Zn	66
13	Zn	68
14	As	75
15	Se	77
16	Se	82
17	Ag	107
18	Ag	109
19	Cd	111
20	Cd	114
21	Sb	121
22	Sb	123
23	Tl	203

24	Tl	205
25	Pb	208
26	U	235
27	U	238
28	Mo	98
29	Ba	135
30	Sr	88

Equipment:

1. Inductively coupled plasma–mass spectrometer. This instrument consists of a mass spectrometer, detector, an ICP source, and mass flow controllers for regulating ICP gas flows, a peristaltic pump for introducing samples, and a computerized data acquisition and instrument control system. An x-y auto sampler may also be used with appropriate control software.
2. Laboratory ware. Precleaned plastic laboratory wares for standard and sample preparations are used. Teflon is preferred for standard preparation and sample digestion; metal-free plastics may be acceptable for internal standards, known addition solutions, etc.
3. Micro pipetts. Sizes from 10 to 100 µl, 100 to 1000 µl, and 1.0 to 10 ml.
4. Analytical balance accurate to 0.001 mg.
5. Sample-preparation apparatus. Hot plates, microwave digesters, and heated sand baths are used.
6. Clean hood.

Reagents:

1. Acids. Ultra-high-purity grade (or equivalent) acids, to prepare standards and process samples. Extreme care is taken when handling acids in the laboratory, to avoid contaminating with trace levels of metals. Nitric acid (HNO_3) concentrated (specific gravity 1.41), nitric acid (1:1), nitric acid, 2 % and 1% (v/v).
2. Reagent water. Metal free water is used for preparing blanks, standards, and samples.
3. Stock standard solutions. High-purity stock solutions are commercially available.
4. Internal standard stock solution. Germanium, indium, lithium, scandium, and thorium are suggested as internal standards. The following masses are monitored: ^{72}Ge, ^{115}In, ^{6}Li, ^{45}Sc, and ^{232}Th. enough internal standard is added to all test samples, standards, and quality control (QC) samples to give a suitable counts/second (cps). Dilution-related errors are minimized by using an appropriately high concentration of internal standard mix solution. Maintain volume ratio for all internal standard additions.
5. Tuning solution. It contains beryllium, cadmium, cobalt, copper, germanium, indium, rhodium, scandium, terbium, thallium, (for sensitivity and stability evaluation), barium (for doubly-charged evaluation), cerium (for oxide evaluation), magnesium (for mass calibration check), and lead (for mass calibration check). Prepare this solution in 2 % HNO_3. This mix includes all common elements used to optimize and tune various

ICP–MS operating parameters. It may be possible to use fewer elements in this solution, depending on the instrument manufacturer's recommendations.

6. Calibration standards. A five-calibration standard is recommended. All calibration standards and blanks are prepared in a matrix of 2 % nitric acid. Add internal standard. (Note: Add the same ratio of internal standard mixture to all standards and blanks).
7. Method blank (MB). A method blank (also known as reagent blank) is a portion of reagent water.
8. Calibration verification standard. Prepare a mid-range standard using different sources than that used for the calibration standards, in 2 % HNO_3, with equivalent addition of internal standard.
9. Calibration verification blank. Use 2 % HNO_3, the same solution as the zero-calibration standard.
10. Laboratory-fortified blank (LFB). It is also known as a blank spike, i.e., a method blank that has been fortified with a known concentration of analyte. It is used to evaluate on going laboratory performance and analyte recovery.

Procedure:

1. Sample preparation. See the above preparation section.
2. Instrument operating conditions. Follow manufacturer's standard operating procedures for initialization, mass calibration, gas flow optimization, and other instrument operating conditions.
3. Analytical run sequence. Make a run sequence, including tuning and optimizing the instrument, checking reagent blanks, calibrating the instrument, verifying the calibration, analyzing samples, and analyzing quality control samples and blanks.
4. Instrument tuning and optimization. Follow manufacturer's instructions for optimizing instrument performance.
5. Instrument calibration. After optimization and tuning, calibrate the ICP–MS using an appropriate range of calibration standards for acceptable calibrations, the regression curves' correlation coefficient is ideally 0.995 or greater. Immediately after calibration, run initial calibration verification standard, acceptance criteria are 10% of known analyte concentration. Next, run initial calibration verification blank.
6. Sample analysis. Ensure that all vessels and reagents are uncontaminated. During the analytical run, include quality-control analyses according to QA/QC protocols. Internal standard recoveries must be between 70 and 125 % of internal standard response in the laboratory-fortified blank; otherwise, dilute sample, add internal standard mixture, and reanalyze. Make known-addition analyses for each matrix in a digestion or filtration batch.

Calculation and correction:

Configure instrument software to report internal standard corrected results. The results are reported in micrograms per liter.

Interferences:

1. Isobars. Isobars are isotopes of different elements that form ions with the same nominal atomic mass units/charge number (m/z) ratio that cannot be resolved by a quadrupole or high-resolution mass spectrometer. Typically, ICP–MS operating software includes all known isobaric interferences and will perform the necessary calculations automatically and use recommended mass.
2. Abundance sensitivity. Is the possibility that the low and high "wings" of any abundant mass peak will contribute to or obscure adjacent masses. Adjust the mass spectrometer resolution and quadrupole bias to minimize these interferences.
3. Polyatomic species. Molecular ion interferences caused by ions with more than one atom that have the same nominal m/z ratio as the isotope of interest. Most of the common molecular ion interferences have been identified (see Table III. 6).
4. Because of the severity of chloride ion interference on important analytes, particularly vanadium and arsenic, hydrochloric acid is not recommended for use in ICP–MS sample preparation.
5. Because most environmental samples contain some chloride ion, correction equations are used for compensation for chloride and the affected masses. Collision cell technology and dynamic reaction cell effectively reduce most polyatomic species to analytically negligible levels in quadrupole-based ICP–MS systems, sometimes avoiding the need for complex correction equations.
6. A high-resolution ICP–MS resolves, but not all, interferences caused by polyatomic ions.
7. Polyatomic interferences are strongly influenced by instrument design and plasma operating conditions; they sometimes can be reduced by carefully adjusting nebulizer gas flow and other instrument operating parameters.

Table (III.6) Polyatomic interferences

S	m/z	Element	Polyatomic interferences	Elemental interferences
1	52	Cr	$^{35}Cl^{16}OH$, $^{40}Ar^{12}C$, $^{36}Ar^{16O}$, $^{37}C^{l15}N$ $^{34}S^{18}O$	$^{104}Pd^{2+}$, $^{104}Ru^{2+}$
2	53		$^{37}Cl^{16}O$, $^{38}Ar^{15}N$, $^{38}Ar^{14}NH$, $^{36}Ar^{16}OH$, $^{40}Ar^{13}C$	$^{106}Pd^{2+}$, $^{106}Cd^{2+}$
3	55	Mn	$^{40}Ar^{15}N$, $^{40}Ar^{14}NH$, $^{39}K^{16}O$, $^{38}Ar^{16}OH$	$^{110}Cd^{2+}$
4	60	Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{43}Ca^{16}OH$	$^{120}Sn^{2+}$, $^{120}Te^{2+}$
5	62		$^{46}Ti^{16}O$, $^{23}Na^{39}K$, $^{46}Ca^{16}O$	$^{124}Te^{2+}$, $^{124}Sn^{2+}$, $^{124}Xe^{2+}$
6	63	Cu	$^{31}P^{16}O_2$, $^{40}Ar^{23}Na$, $^{47}Ti^{16}O$, $^{23}Na^{40}Ca$, $^{46}Ca^{16}OH$	$^{126}Te^{2+}$, $^{126}Xe^{2+}$
7	65		$^{49}Ti^{16}O$, $^{32}S^{16}O_2H$, $^{40}Ar^{25}Mg$, $^{40}Ca^{16}OH$, $^{36}Ar^{14}N_2H$	$^{130}Te^{2+}$, $^{130}Xe^{2+}$, $^{130}Ba^{2+}$
8	66	Zn	$^{50}Ti^{16}O$, $^{34}S^{16}O_2$, $^{33}S^{16}O_2{}^1H$, $^{32}S^{16}O^{18}O$, $^{32}S^{17}O_2$	$^{132}Xe^{2+}$, $^{132}Ba^{2+}$
9	68		$^{36}S^{16}O_2+$, $^{34}S^{16}O^{18}O^+$, $^{40}Ar^{14}N_2{}^+$, $^{35}Cl^{16}O^{17}O^+$, $^{34}S_2$	$^{136}Ba^{2+}$, $^{136}Xe^{2+}$, $^{136}Ce^{2+}$
10	75	As	$^{40}Ar^{35}Cl$, $^{59}Co^{16}O$, $^{36}Ar^{38}ArH$, $^{38}Ar^{37}Cl$,	$^{150}Sm^{2+}$, $^{150}Nd^{2+}$

			$^{36}Ar^{39}K$	
11	78	Se	$^{38}Ar^{40}Ar$, $^{62}Ni^{16}O$	^{78}Kr, $^{156}Gd^{2+}$, $^{156}Dy^{2+}$
12	82		$^{40}Ar^{2}H2$, $^{66}Zn^{16}O$	^{82}Kr, $^{164}Dy^{2+}$, $^{164}Er^{2+}$
13	95	Mo	$^{79}Br^{16}O$	
14	98		$^{82}Kr^{16}O$, $^{82}Se^{16}O$	^{98}Ru
15	111	Cd	$^{95}Mo^{16}O$, $^{94}Zr^{16}OH$, $^{39}K_2{}^{16}O^{2}H$	
16	114		$^{98}Mo^{16}O$, $^{98}Ru^{16}O$	^{114}Sn
17	201	Hg	$^{186}W^{16}O$	
18	202			
19	206	Pb	$^{190}Pt^{16}O$	
20	207		$^{191}Ir^{16}O$	
21	208		$^{192}Pt^{16}O$	
22	74	Ge	$^{34}S^{40}Ar$, $^{37}Cl^{37}Cl$, $^{58}Fe^{16}O$, $^{58}Ni^{16}O$	^{74}Se, $^{148}Nd^{2+}$, $^{148}Sm^{2+}$
23	103	Rh	$^{63}Cu^{40}Ar$, $^{87}Sr^{16}O$	$^{206}Pb2+$
24	193	Ir	$^{177}Hf^{16}O$	
25	209	Bi	$^{193}Ir^{16}O$	

8. Doubly-charged ions. Some elements (e.g., barium and strontium) form significant levels of M2 ions under normal plasma conditions. The M2 ions occur in the mass spectrum at M/2 and, in the case of Ba and Sr, will interfere with some isotopes of zinc and calcium, respectively.
9. Physical interferences. These include differences in viscosity, surface tension, and dissolved solids between samples and calibration standards. To minimize these effects, analytical samples should not contain more than 0.5 % of dissolved solids. Dilute water and wastewater samples with higher dissolved solids levels are diluted before analysis. Internal standards are used to correct for physical interferences, provided that their analytical behavior is comparable to the elements being determined.
10. Memory interferences. These occur when analytes from a previous sample or standard are measured in the current sample. Long enough rinse (flush) between samples is used to minimize such interferences.
11. Ionization interferences. These result when moderate (0.1 % to 1 %) amounts of a matrix ion change the analyte signal. This effect, which usually reduces the analyte signal, is also known as suppression and is corrected by using internal standardization techniques.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 3125(B).

(III.11) Alkali Metals and Alkaline Earth Metals

(Ion-Chromatography)

Principle:

A water sample is injected into a stream of suitable eluent and passed through an exchange column where the ions of interest are separated. The separated ions are passed through a suppressor column to convert the separated ions to their highly conductive forms and convert the eluent into weakly conductive species. The separated ion is determined using conductometric detector. This method simultaneously determines the concentrations of lithium, sodium, ammonium, potassium, magnesium, and calcium cations. The data is recorded, integrated and the final results are reported by computer. This automated method is applicable for surface, saline, underground, drinking, domestic and industrial wastewaters. The method is also viable for aqueous solutions of soil extracts and finds wide applications for the determination of anions.

Equipment

1. Ion chromatograph (IC).
2. Assorted sizes of class A pipettes and volumetric flasks.

Table (III.7) Chromatographic operational conditions

Chromatographic conditions	
Column	IonPac CS12A analytical, (4 × 250 mm)
Guard column	IonPac CG12A guard, (4 × 50 mm)
Suppressor	CSRS® ULTRA, ∏4 mm
Detection	Conductivity detector
Eluent generator	59 mM MSA
Sample loop	25 µl
Pump flow rate	1.0 ml/min
Backpressure:	~1490 psi
Runtime	12 min

Table (III.8) Retention times expected with high concentration range standards

Analyte	Concentration of mixed standard, mg/l	Retention time, min
Lithium	10	3.47
Sodium	60	4.07
Ammonium	20	4.63
Potassium	20	5.88
Magnesium	20	8.63
Calcium	60	10.97

Reagents:

1. Glassware cleaning. In addition to normal cleaning operations, acid wash (10 % hydrochloric acid) glassware used for reagent preparation followed by at least five rinses with demonized water.
2. Deionized water. Water with conductivity more than 18 MΩ/cm, used immediately after purification
3. Chemical purity requirements. Reliable, consistent and accurate results require eluents that are free of ionic impurities. Chemicals, solvents and deionized water used to prepare eluents and standards must be of the highest purity available. Low trace impurities and low particle levels in eluents also help to protect the ion exchange columns and system components.
4. Preparation of standards and reagents. For several of the analytes of interest, 1000 mg/l standard solutions are available from Dionex and other commercial sources. When commercial standards are not available, 1000 mg/l standards can be prepared by dissolving the appropriate amounts of the required analytes in 1000 ml of deionized water.

a. Lithium standard, 1000 mg/l as Li+

Lithium chloride (LiCl)…… 6.107 g
Deionized water ..... 1000 ml
Preparation*:*

- Pre-dry approximately 7 g of lithium chloride at 104±1 °C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 6.107±0.001 g of the pre-dried salt into a one litre volumetric flask and dilute to 1000 ml with demonized water and mix.
- Store the solution in one litre amber plastic bottle. The solution is stable for at least one month. So, the standard is prepared monthly.

b. Sodium standard, 1000 mg/l as Na+

Sodium chloride (NaCl)..... 2.5414 g
Demonized water ..... 1000 ml

Preparation:

- Pre-dry approximately 3 g of sodium chloride at 104±1°C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 2.5414±0.001 g of the pre-dried salt into a one litre volumetric flask and complete to the mark with deionized water and mix.
- Store the solution in an amber plastic bottle and stored at 4±2 °C. The solution is stable for at least one month.

c. Ammonium sstandard, 1000 mg/l as NH4+

Ammonium chloride (NH_4Cl)……. 2.9654 g
Demonized water……. 1000 ml

Preparation*:*

- Pre-dry approximately 4 g of ammonium chloride at 104±1°C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 2.9654±0.001 g of the pre-dried salt into a one litre volumetric flask and dilute to the mark with deionized water and mix. This stock is prepared every month and stored at 4±2 °C.

d. **Potassium standard, 1000 mg/l as K+**

Potassium chloride (KCl).....1.9068 g

Demonized water .....1000 ml

Preparation:

- Pre-dry approximately 3 g of potassium chloride at 104±1 °C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 1.9068±0.001 g of the pre-dried salt into a one litre volumetric flask and dilute to the mark with deionized water and mix. This stock solution is prepared every month and stored at 4±2 °C.

e. Magnesium standard, 1000 mg/l as Mg^{2+}

Magnesium chloride ($MgCl_2$).....3.9173 g

Deionized water.....1000 ml

Preparation*:*

- Pre-dry approximately 5 g of magnesium chloride at 104±1°C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 3.9173±0.001 g of the pre-dried salt into a one litre volumetric flask and dilute to the mark with deionized water and mix. This stock solution is prepared monthly and stored at 4±2 °C.

f. **Calcium standard, 1000 mg/l as Ca^{2+}**

Calcium chloride ($CaCl_2$)..... .. 2.7692 g

Deionized water.....1000 ml.

Preparation:

- Pre-dry approximately 3 g of calcium chloride at 104±1 °C for two hours, and allow cooling to room temperature in a desiccator for one hour.
- Place 2.7692±0.001 g of the pre-dried salt into a one litre volumetric flask and dilute to the mark with deionized water and mix. This stock solution is prepared monthly.

5. Working standard solutions. Composite working standard solutions at specified analyte concentrations are prepared from the 1000 mg/l stock solutions (Table III. 8), stored at 4±2 °C and prepared monthly. This standard is commonly used with CS12A analytical column and CG12A guard column. All stock standards for cations are stable for at least 6 months when stored at 4±2 °C. Dilute working standards should be prepared monthly.

Table (III.9) Standard solutions used for instrument calibration

Analyte	Volume of stock solution (in ml) used to prepare high-range standard	Concentration, mg/l		
		High-range standard	Intermediate- range standard	Low- range standard
Lithium	10	10.0	5.0	2.5
Sodium	60	60.0	30.0	15.0
Ammonium	20	20.0	10.0	5.0
Potassium	20	20.0	10.0	5.0
Magnesium	20	20.0	10.0	5.0
Calcium	60	60.0	30.0	15.0

- Three levels of calibration standards are used in this method to cover the expected concentrations found in environmental samples.

*Volumes of each stock solution (1.00 ml ≡ 1.00 mg) that are combined in a Class A volumetric flask and diluted to 1 liter to prepare the high-range calibration standard.

Procedure:

A. Sample holding and preservation:

Water samples collected for analysis by IC should be collected in plastic containers, such as polystyrene or polypropylene bottles, as glass bottles can contribute ionic contamination when performing trace analysis. The bottles should be thoroughly rinsed with reagent-grade water before use. The minimum quantity required to analyse one sample is 100 ml. Sample preservation requirements and holding times for cations determined by IC are listed in Table (III.10).

Table (III.10) Sample preservation and holding times for some cations determined by IC

Analyte	Preservation	Holding time, days
Lithium (Li^+)	Filtration	42
Sodium (Na^+)	Filtration	42
Ammonium (NH_4^+)	Filtration, cool to 4 ± 2 °C.	7
Potassium (K^+)	Filtration	42
Magnesium (Mg^{2+})	Filtration	42
Calcium (Ca^{2+})	Filtration	42

***B. Sample preparation**:*

Water samples, collected for IC analysis, require little or no sample pre-treatment. Drinking-water samples, for instance, typically require no pre-treatment other than filtration through a 0.45 µm filter to remove particulates. Higher ionic strength water samples, e.g. wastewater, often only require dilution (and filtration) to bring the analytes of interest into the working range of the method. However, solid samples, such as soils and sludge, are not directly amenable to IC analysis and require additional sample pre-treatment and soil extracts.

C. Sample measurements:

1. Set-up the Dionex chromatograph or equivalent system according to the equipment manuals.
2. Run the chromatograph software (Chromeleon® 6.8 Chromatograph Workstation). The Dionex analytical method file name is "System 01" for cations and anions methods. Select cations method (using CS12A Analytical column) and fill the sequence starting blank, standards, QC samples and samples. Run a test from sequence control and start batch.
3. Check the retention times for each analyte. A reduced retention time is an indication of a dirty column. If the reduced retention time is not pronounced, change the method to account for new retention times. To clean column refer to the appropriate column instruction manual.
4. If the concentration of an analyte is greater than 15 % of the highest standard, dilute the sample and rerun.
5. For each run monitor cation sensitivities as determined by analysis of calibration standards. Sensitivities should not deviate by more than 10 % from expected values. Check calibration stability by analysing a check standard (QC sample) every 10-sample tube and after all the samples.
6. If results are not within 10 % of the expected values, correct the problem and re-analyse the affected samples.

Table (III. 11) Volumes of the used stock solution

Analyte	Volume of stock solution (in ml) used to prepare quality control samples	Concentration, mg/l	
		QC1	QC2
Lithium	5.0	0.50	5.00
Sodium	50.0	5.00	50.00
Ammonium	10.0	1.00	10.0
Potassium	20.0	2.00	20.00
Magnesium	20.0	2.00	20.00
Calcium	50.0	5.00	50.00

Quality control samples preparation:

Volumes of each stock solution (1.00 ml ≡ 1.00 mg) are combined in a class A volumetric flask and diluted to 1 liter to prepare quality control sample (QC2). A 100 ml aliquot of (QC2) is diluted to 1liter to prepare (QC1). The stability of QC samples is one month when stored at 4 ± 2°C.

Quality control:

1. Results for accuracy of quality control samples must be within supplier designated limits or, where these are not available, results must be within 10% of expected values.
2. Analyse at least 10 % of the samples in replicate, with a minimum of one sample per set. The % RSD of duplicate results not close to the detection limit must not exceed 20 %.

3. Analyse at least one blank with each batch of samples. Blank results must be less than the detection limits. Investigate occurrences of positive blanks and rerun sample analysis after solving problem.
4. Duplicate sample analyses should be performed on about 5 % of routinely analyzed samples.
5. Analyse a check solution or standard after every ten sample vials. Check the slope of the calibration curve. The correlation coefficient must be greater than 0.99.
6. Create a control chart using quality control samples results (QCS) (see section X). Excel program can be used.

Method detection limit (MDL):

MDL must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where:

t = student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = standard deviation of the replicate analyses.

MDL should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

Safety and precautions:

There are no special safety precautions for this method. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of "Safety Data Sheets (SDS)" should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

Interferences:

1. Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of cations can interfere with the peak resolution of an adjacent cation. Sample dilution and/or fortification can be used to solve most interference problems associated with retention times.
2. Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artefacts or elevated baseline in ion chromatograms.
3. Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage of the

instrument columns and flow systems. So filtered the samples through 0.45-µm pore-size by Whatman paper or equivalent filters.

Calibration and standardization:

1. For each analyte of interest, prepare calibration standard at a minimum of three concentration levels.
2. If a sample analyte concentration exceeds the calibration range, the sample may be diluted to fall within the measurable range. If this is not possible, three new calibration concentrations must be chosen.
3. The calibration curve must be verified on each working day, or whenever the cation eluent is changed, and after every 20 samples.
4. If the response or retention time for any analyte varies from the expected values by more than ±10 %, the test must be repeated, using fresh calibration standards. If the results are still more than ±10 %, a new calibration curve must be prepared for that analyte

Data analysis and calculations:

1. Prepare a calibration curve for each analyte by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.
2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Report results in mg/l.

Method validation:

Precision and accuracy. For CS12A cation analytical column, the cation levels and average concentration to be measured are given in Table (III.12). Accuracy, standard deviation (precision) and uncertainty are given in Table (III.13).

Table (III.12) Cations levels and average concentration

Parameter	Level	Average, mg/l
Lithium	10	9.95
Sodium	60	59.20
Ammonium	20	19.86
Potassium	20	19.77
Magnesium	20	19.92
Calcium	60	59.87

Table (III.13) Accuracy, standard deviation (precision) and uncertainty

Element	Designed value, mg/l	Range value	Average value	Accuracy, %	Bias	Standard deviation	Uncertainty	RSD, %
Li	0.50	0.5 -	0.5118	102.371	0.00	0.00756	±0.527	1.477
	5.00	5.19 –	5.232	104.657	0.00	0.03946	±0.0789	0.754

Na	5.00	5.04 –	5.152	103.057	0.00	0.06775	±0.1355	1.315
	50.00	49.20	49.708	99.417	0.00	0.3324	±0.6650	0.669
NH_4	1.00	0.98 –	1.0828	108.286	0.00	0.09340	±0.1868	8.625
	10.00	9.97 –	10.251	102.514	0.00	0.25116	±0.5023	2.450
K	2.00	2.00 –	2.064	103.214	0.00	0.04756	±0.0951	2.304
	20.00	20.50	20.642	103.214	0.00	0.1618	±0.323	0.784
Mg	2.00	1.93 –	2.065	103.286	0.00	0.0725	±0.145	3.512
	20.00	20.40	20.60	103.000	0.00	0.1527	±0.305	0.742
Ca	5.00	4.60 -	4.948	98.971	0.00	0.2021	±0.404	4.086
	50.00	49.99	50.427	100.854	0.00	0.3222	±0.644	0.639

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 [Method 4110 (C)].

(IV) Aggregate Organic Constituents

(IV.1) Chemical Oxygen Demand (COD)
(Titrimetry-Open Reflux Method)

Principle:

This method is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant and expressed as milligrams of O_2/liter. Most types of organic matter are oxidized by boiling with a mixture of potassium dichromate and sulfuric acid in the presence of mercuric sulfate catalyst and silver sulfate to precipitate chloride ion, if present. After digestion, the remaining unreduced potassium dichromate is titrated with ferrous ammonium sulfate using ferroin indicator and the oxidizable organic matter is calculated in terms of oxygen equivalent. This parameter is a measure of pollutants in waste water, natural water, drinking, brackish, and saline water.

Equipment:

Reflux apparatus consisting of 500 ml flask with ground glass neck and equivalent condenser with ground glass joint and a hot plate.

Reagents:

1. Standard potassium dichromate, 0.25 N. Dissolve 12.259 g potassium dichromate ($K_2Cr_2O_7$), previously dried at 103°C for 2 hours, in distilled water and dilute to 1 litre, the equivalent concentration is 0.2500N. To prepare 0.025 N solution, take 100 ml of 0.25 N $K_2Cr_2O_7$ solution and dilute to 1000 ml with distilled water.

2. Sulfuric acid solution: Add 22 g silver sulfate ($AgSO_4$) to (4 Kg) of concentrated H_2SO_4. Keep overnight for dissolution. Shake well after dissolution.
3. Standard ferrous ammonium sulfate solution (0.25 M) (FAS). Dissolve 98 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 ml of concentrated H_2SO_4 and dilute to 1 litre. Standardize this solution daily against a standard $K_2Cr_2O_7$.
4. Standardization of ferrous ammonium sulfate is carried out as follows. Dilute 25 ml standard $K_2Cr_2O_7$ solution to about 100 ml. Add 30 ml concentrated H_2SO_4 and cool. Add 3-4 drops of ferroin indicator and titrate with ferrous ammonium sulfate solution till the color changes to wine red. The normality of $Fe(NH_4)_2(SO_4)_2$ solution is given by the following formula:

$$\text{Normality of } Fe(NH_4)_2(SO_4)_2 \text{ solution} = \frac{25 * 0.25}{Fe(NH_4)_2(SO_4)_2, \text{ml}}$$

5. Ferroin indicator. Dissolve 1.485 g of 1,10-phenanthroline monohydrate, and 695 mg $FeSO_4.7H_2O$ in water and dilute to 100 ml.
6. Mercury sulfate ($HgSO_4$), crystals.

Procedure:

A. Blank preparation:

Place 50 ml distilled water, 25 ml 0.025 M $K_2Cr_2O_7$, glass beads and 75 ml of concentrated H_2SO_4 in a flask.

B. Sample preparation:

1. Place 50 ml sample (for samples with high COD, use smaller sample portion and diluted to 50 ml), add 25 ml 0.025 M $K_2Cr_2O_7$, glass beads and add very slowly 75 ml of concentrated H_2SO_4 in the reaction flask.
2. Wash the condenser with distilled water
3. Attach to a reflux condenser and turn on cooling water.
4. Put the bottles containing the blank and test sample on hot plate or heating mantle.
5. Reflux for 2 h. (calculate the 2 h from the samples start boiling).
6. Cool the flask for 1 h.
7. Titrate excess $K_2Cr_2O_7$ with 0.1N ferrous ammonium sulfate using ferroin indicator. The end point is detected by the appearance of the first reddish brown color.

C. Measurement of high-COD samples:

1. Samples with COD > 900 mg/l are analyzed by using smaller sample portion, and diluted to 50 ml.
2. Add 1 g $HgSO_4$, 1 g Ag_2SO_4 and glass beads. Add very slowly 5 ml of concentrated H_2SO_4 to dissolve $HgSO_4$, and Ag_2SO_4.
3. Add 25 ml of 0. 25 M $K_2Cr_2O_7$ and slowly add 70 ml of concentrated H_2SO_4 to the reaction flask.
4. The same steps are followed, but titration with 0.25 N ferrous ammonium sulfate using ferroin indicator.

Calculation:

Normality of ferrous ammonium sulfate:

$$N \times V = N` \times V`$$

N= normality of $K_2Cr_2O_7$.

V= volume of $K_2Cr_2O_7$ (10 ml).

N`= normality of ferrous ammonium sulfate.

V`= volume of ferrous ammonium sulfate consumed in titration.

$$COD, mgO_2 / L = \frac{(A-B) \times N \times 8000}{\text{Volume of sample}}$$

A = volume (ml) of ferrous ammonium sulfate used for blank.

B = volum (ml) of ferrous ammonium sulfate used for sample.

N = normality of ferrous ammonium sulfate.

8000 = milliequivalent weight of oxygen x 1000 ml/l.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017(open reflux, titrimetric method 5220-B).

(IV.2) Chemical Oxygen Demand (COD)

(Titrimetry - Closed Reflux Method)

Principle:

Most types of organic matter are oxidized by boiling with a mixture of chromic and sulfuric acids. The test sample in strongly acid solution is refluxed with a known excess of potassium dichromate ($K_2Cr_2O_7$) in a closed vial. After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed in the oxidation process as previously described.

Equipment:

1. Digestion vessels**.** Preferably use borosilicate culture tubes, 16 × 100-mm, 20 ×150 - mm, or 25 × 150-mm, with TFE-lined screw caps. Alternatively, use borosilicate ampules, 10-ml capacity, 19- 20-mm diam.
2. Block heater. Heated metal block or similar device to operate at 150±2 °C, with holes to accommodate digestion vessels. Use of culture tubes probably requires the caps to be outside the vessel to protect caps from heat.
3. Microburette.
4. Ampule sealer: Use only a mechanical sealer to insure strong, consistent seals.

Reagents:

1. Standard potassium dichromate digestion solution, 0.01667 M. Add to about 500 ml distilled water, 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 ml concentrated H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 ml.
2. Sulfuric acid reagent. Add Ag_2SO_4, reagent or technical grade, crystals or powder, to concentrated H_2SO_4 to obtain 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand for 1 to 2 days to dissolve, and mix.
3. Ferroin indicator solution. Dissolve 1.485 g of 1,10-phenanthroline mono-hydrate and 695 mg $FeSO_4$ $6H_2O$ in distilled water and dilute to 100 ml.
4. Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10 M. Dissolve 39.2 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 ml concentrated H_2SO_4, cool, and dilute to 1000 ml with distilled water.
5. Standardize the ferrous solution daily against standard $K_2Cr_2O_7$ digestion solution as follows: Pipet 5.00 ml of the digestion solution into a small beaker. Add 10 ml reagent water, 1 to 2 drops dilute ferroin indicator and titrate with FAS titrant.

$$\text{Molarity of FAS solution} = \frac{\text{Volume 0.01667 M } K_2Cr_2O_7 \text{ solution titrated, ml}}{\text{Volume FAS used in titrati on, ml}} \times 0.1000$$

6. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$. Lightly crush and dried KHP to constant weight at 110°C. Dissolve 425 g in distilled water and dilute to 1000 ml KHP has a theoretical COD of 1.176 mg O_2/mg and 500 µg O_2/ ml.

N.B. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth.

Procedure:

1. Refer to Table (IV.1) for proper sample and reagent volumes.
2. Make volumetric measurements as accurate as practical; use class A volumetric glasswares. The most critical volumes are of the sample and digestion solution.
3. Use a micro-burette for titrations. Measure H_2SO_4 to ±0.1 ml. The use of hand-held pipettes with non-wetting (polyethylene) pipet tips is practical and adequate.
4. Place the test sample in a culture tube or ampule and add the digestion solution.
5. Carefully add sulfuric acid reagent down inside of the sample tube, so an acid layer is formed under the sample-digestion solution layer.
6. Tightly cap the tubes or seal ampules, and invert each several times to mix completely.
7. Wear face shield and protect hands from heat produced when contents of vessels are mixed.
8. Mix thoroughly before applying heat to prevent local heating of the vessel bottom and possible explosive reaction.
9. Place tubes or ampules in block digester preheated to 150 °C and reflux for 2 h behind a protective shield. These sealed vessels may be under pressure from gases generated during digestion.
10. If sulfuric acid is omitted or reduced in concentration, very high and dangerous pressures will be generated at 150 °C.
11. Cool to room temperature and place the vessels in test tube rack.
12. Some mercuric sulfate may precipitate out but this will not affect the analysis.
13. Remove culture tube caps and add small TFE-covered magnetic stirring bar. If ampules are used, transfer contents to a larger container for titration.
14. Add 0.05 to 0.10 ml (1 to 2 drops) ferroin indicator and stir rapidly on a magnetic stirrer while titrating with standardized 0.10 M FAS. The end point is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes.
15. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample (Table IV. 1).

Calculation:

$$COD, mgO_2 / L = \frac{(A-B)\times M \times 8000}{\text{Volume of sample}}$$

where:

A = ml FAS used for blank.

B = ml FAS used for sample.

M = molarity of FAS.

8000 = milliequivalent weight of oxygen × 1000 ml/l.

Non homogeneous test sample requires multiple determinations for accurate analysis.

All results should agree within ± 5 % of their average, unless the conditions of the sample dictates otherwise.

Table (IV.1) Volumes of the samples versus the reagents

Digestion vessels	Sample, ml	Digestion solution, ml	Sulfuric acid reagent, ml	Total final volume,ml
10×100 mm	2.5	1.5	3.5	7.5
20×150 mm	5.00	3.00	7.0	15.0
25x150 mm	10.00	6.00	14.0	30.0
Standard 10-ml ampules	2.5	1.5	3.5	7.5

Precautions:

1. Wear face and hand protection when handling the procedures.
2. Do not use the test for samples containing more than 2000 mg Cl^-/l.
3. Halide interferences (in sea water) may be removed by precipitation with silver ion and filtration before digestion.
4. Wash culture tubes and caps with 20 % H_2SO_4 before first use to prevent contamination.

Interferences:

1. The most common interfering species is the chloride ion. Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide, iodide, and any other reagent interact with silver ion and similarly interfere.
2. Such interferences are negative in that they tend to restrict the oxidizing action of the dichromate ion itself.
3. Under the rigorous digestion procedures for COD analyses, chloride, bromide, or iodide react with dichromate to produce the elemental form of the halogen and the chromic ion, thus resulting in positive errors.
4. The difficulties caused by the presence of the chloride can be overcome largely, but not completely, by using excess of mercuric sulfate ($HgSO_4$) before the refluxing procedure.
5. Although 1 g $HgSO_4$ is specified for 50 ml sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/l, as long as a 10:1 weight ratio of $HgSO_4$: Cl is maintained.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Method 5220 C. closed reflux, titrimetric method).

(IV.3) Chemical Oxygen Demand (COD)

(Spectrophotometry - Vial Reflux Method)

Principle:

The sample is heated for two hours with excess potassium dichromate as previously outlined. The reagent contains silver and mercury ions to catalyze the reaction and complex chloride ions, respectively. Oxidizable organic compounds reduce dichromate ion ($Cr_2O_7^{2-}$) to green chromous (Cr^{3+}) ion. Either the amount of dichromate remaining or the amount of chromus ion produced is colorimetrically measured to determine the oxygen demand.

Equipment:

1. COD reactor (block heater operates at 150±2 °C).
2. COD vial adapter, DR/2010.
3. Micro pipette 2 ml, class A.
4. Test tube rack.
5. Spectrophotometer, for use at 600 nm and/or 420 nm**.**

Reagents:

1. Digestion solution, high range: Add to about 500 ml distilled water 10.216 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 ml concentrated H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 ml.

2. Digestion solution, low range: Prepare as above, but use only 1.022 g potassium dichromate.
3. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to concentrated H_2SO_4 at the rate of 5.5 g Ag_2SO_4/Kg H_2SO_4. Let stand 1 to 2 days to dissolve and mix.
4. Potassium hydrogen phthalate (KHP) standard: Lightly crushed and dried KHP to constant weight at 110 °C for 2 h is used. Dissolve 425 mg in (organic free) deionized water and dilute to 1000 ml. KHP has theoretical COD of 500 mg/l O_2. This solution is stable when refrigerated, but not indefinitely. Twice monthly preparation usually is satisfactory.

Procedure:

1. Measure suitable volume of the test sample and reagents into tube or ampule as indicated in Table (IV.1). Prepare, digest, and cool samples, blank, and one or more standards.

2. It is critical that the volume of each component be known and that the total volume is the same for each reaction vessel. Homogenize 100 ml of the sample for 30 seconds by shaking.
3. Cool sample to room temperature slowly to avoid precipitate formation.
4. Once samples are cooled, vent, if necessary, to relieve any pressure generated during digestion.
5. Mix contents of reaction vessels to combine condensed water and dislodge insoluble matter.
6. Let suspended matter settle and ensure that optical path is clear.
7. Measure absorption of each sample blank and standard at selected wavelength (420 nm or 600 nm).
8. At 600 nm, use an undigested blank as reference solution. Analyze a digested blank to confirm good analytical reagents and to determine the blank COD; subtract blank COD from sample COD. Alternately, use digested blank as the reference solution once it is established that the blank has a low COD.
9. At 420 nm, use reagent water as a reference solution. Measure all samples, blanks, and standards against this solution.
10. The absorption measurement of an undigested blank containing dichromate, with reagent water replacing sample, will give initial dichromate absorption.
11. Any digested sample, blank, or standard that has a COD value will give lower absorbance because of the decrease in dichromate ion. Analyze a digested blank with reagent water replacing sample to ensure reagent quality and to determine the reagents' contribution to the decrease in absorbance during a given digestion.
12. The difference between absorbances of a given digested sample and the digested blank is a measure of the sample COD.
13. When standards are run, plot differences of digested blank absorbance and digested standard absorbance versus COD values for each standard.
14. Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents to cover each concentration range.
15. Make up to volume with reagent water; use same reagent volumes, tube, or ampule size, and digestion procedure as for samples.

Calculation:

If samples, standards, and blanks are run under the same conditions of volume and optical path length, calculate COD as follows:

$$\text{COD as mg } O_2/l = \text{mg } O_2 \text{ in final volume x } 1000 \text{ /ml sample}$$

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5220 D).

(IV.4) Biochemical Oxygen Demand (5 Day BOD)

(Titrimetry-Winkler Method)

Principle:

It is the amount of oxygen consumed by bacteria for oxidation of organic matter. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 - 7.5. The method consists of filing an airtight bottle of specified size with dilute and seeded sample, to over flowing, and incubating it at the specified temperature (20ºC) for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. BOD is used as a measure of the oxygen equivalent to the organic matter content of a sample that is susceptible to oxidation by bacteria and as a measurement of pollutants in waste water and natural water.

Equipment:

1. Incubation BOD (Winkler) bottles.
2. Air incubator, thermostatically controlled at 20±1ºC, excludes all light to prevent possibility of photosynthetic production of DO.

Reagents:

1. Manganous sulfate solution. Dissolve 480 g $MnSO_4.4H_2O$ or 400 g $MnSO_4.2H_2O$ or 364 g $MnSO_4.H_2O$ in distilled water and dilute to 1000 ml. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.
2. Alkaline potassium iodide solution. Dissolve 150 g KI + 700 g KOH in distilled water and complete to 1000 ml distilled water. Or dissolve 135 g NaI + 500 g NaOH in distilled water and complete to 1000 ml with distilled water.
3. Starch. Dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid as a preservative, in 100 ml hot distilled water.
4. Standard sodium thiosulfate titrant (0.025 N). Dissolve 6.205 g of $Na_2S_2O_3.5H_2O$ in distilled water. Add 1.5 ml of 6 N NaOH or 0.4 g solid NaOH and dilute to 1000 ml with distilled water. Standardize using standardization solution.
5. Standardization solution. A 10 ml aliquot of potassium dichromate solution 0.025 N, 90 ml distilled water, 2 ml (1:1) HCl and 2 g KI are mixed and left in dark for about 10 min.

6. Acid and alkali solutions. Add 28 ml of concentrated sulfuric acid to distilled water slowly and while stirring. Dilute to 1000 ml with distilled water. Dissolve 40 g sodium hydroxide in distilled water, dilute to 1000 ml with distilled water.
7. Sodium sulfite solution. Dissolve 1.575 g Na_2SO_3 in 1000 ml distilled water. This solution is not stable and should be prepared daily.

Procedure:

A. Preparation of seed suspension:

1. It is necessary to have present in each BOD bottle a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample.
2. Some samples e.g. untreated industrial wastes, disinfected wastes, high temperature wastes, wastes having pH values less than 6 or greater than 8, or wastes stored more than 6 hours after collection do not contain a sufficient microbial population.
3. Seed such samples by adding a population of suitable micro-organisms.
4. Use supernatant from settled domestic waste water, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, un disinfected effluent, or receiving water from below the point of discharge.

B. Method of measurements:

1. Adjust a specified volume of the sample at pH 6.5-7.5.
2. De-chlorinate a specified volume of the sample if present.
3. Adjust a specified volume of the sample at temperature 20±1 ºC and aerate by mixing using two beakers.
4. Fill two bottles with aerated sample till over flow in Winkler bottles.
5. Remove air bubbles by using glass rod.
6. Make two groups of bottles, each group conation one bottle of the sample (initial group, final group).
7. Incubate the final group at 20 ºC for 5 days and immediately add 2 ml manganous sulfate and 2 ml alkaline KI to the initial group and leave for 5 mins.
8. Make sure that the manganous hydroxide precipitate settled down in the bottle, then add 2 ml sulfuric acid. Close the bottle and shake till complete dissolution of the precipitate.
9. Transfer 200 ml of the solution from the bottle to a conical flask and titrate against sodium thiosulfate using starch indicator (the color changes from blue to colorless).
10. After 5 days, repeat the same steps with the final group.

Calculation:

. When the BOD has been determined in an undiluted sample:

BOD_5 at 20ºC = initial DO – final DO x dilution (if you make dilution).

2. When the BOD has been determined in a diluted sample:

BOD, (mg O_2/l) = DO, (mg/l) before incubation - DO (mg/l) after incubation.

2. BOD without applying seed correction:

$$BOD,(mg,O_2/l)=\frac{[(D1-D2)-(B1-B2)]\times 100}{\%\,\text{sample}}$$

3. *BOD with applying seed correction:*

$$BOD,(mg,O_2/l)=\frac{[(D1-D2)-(B1-B2)]\times 100}{\%\,\text{sample}}-SF$$

where:
D1 = DO of diluted sample on day 0.
D2 = average DO of diluted sample on day 5.
B1 = average DO of dilution blank on day 0.
B2 = average DO of dilution blank on day 5.
S = BOD of seed control.
F = ratio of seed in sample to seed in seed control.
SF = seed correction.

C. BOD with volume correction:

This correction is applied in case of low BOD samples, where the sample is incubated in a larger proportion of the dilution. In such cases the fraction of water used for dilution in the test is much less than virtually 100 %. The O_2 consumption due to the addition of dilution water (blank) should reflect the added fraction.
The following modified equation is to be used for applying this correction:

D. BOD without applying seed correction:

$$BOD,(mg,O_2/l)=\frac{[(D1-D2)-(B1-B2)\times A]\times 100}{\%\,\text{sample}}$$

E. BOD with applying seed correction:

$$BOD,(mg,O_2/l)=\frac{[(D1-D2)-(B1-B2)\times A]\times 100}{\%\,\text{sample}}-SF$$

$$A=\frac{100-\%\,\text{sample}}{100}$$

where :
A = the fraction of dilution water added.

Precautions:

1. If residual chlorine is present, dechlorinate sample. In some samples chlorine not dissipate within 1 to 2 h of standing in light. This dissipation often occurs during sample transport and handling.

2. Some samples may be sterile, and will need seeding, but seeding is not continuously required.
3. The presence of heavy metals or other toxic materials such as residual chlorine are other sources of interference in this test.
4. Compounds which are interfering in the BOD test are the same as those Interfering in the determination of dissolved oxygen.
5. The order of adding the nutrient solutions should be: phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride.
6. After aerating the dilution water, leave to stabilize at 20 °C for at least one day.
7. While filling BOD bottles avoid entrapping of bubbles.
8. Wherever necessary, apply seed and volume corrections.
9. Any stock solution showing signs of precipitates or growth, should be discarded.
10. Water from copper stills should not be used, since copper inhibits biochemical oxidation.

Interferences

1. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding Na_2SO_3 solution.
2. Add 10 ml of (1+1) acetic acid or (1+50) H_2SO_4, and 10 ml potassium iodide solution to 1000 ml sample and titrate with Na_2SO_3 solution to the starch-iodine end point.
3. Add to neutralized sample a volume of Na_2SO_3 solution equivalent to the volume determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (Note: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramines compounds that may be present in chlorinated samples). Do not chlorinated/de-chlorinated samples without seeding.
4. Samples containing DO concentration above saturation at 20 ºC may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturation by bringing sample to about 20±3 ºC in partially filled bottle agitating by vigorous shaking or by aerating with clean, filtered compressed air.
5. Hydrogen peroxide remaining in samples from some industrial bleaching processes such as those used in paper mills and textile plants causes supersaturated oxygen levels in samples collected for BOD testing. Mix samples vigorously in open containers for sufficient time to allow the hydrogen peroxide to dissipate before setting up BOD tests. Check adequacy of peroxide removal by observing dissolved oxygen concentrations over time during mixing or by using peroxide-specific test strips. Mixing times can vary from 1 to 2 h depending on the amount of hydrogen peroxide present.
6. The peroxide reaction can be considered complete when the DO no longer increases during a 30 min period without mixing.

7. When effluent or mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended.
8. Do not use seed from effluent that have been disinfected by chlorine or other means. Do not filter seed source; filtering removes the seed micro-organisms.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 405.1).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5210 B).

(IV.5) Biochemical Oxygen Demand (*5-Day* BOD)

(Electrometry - Oxygen Electrode)

Principle:

The biochemical oxygen demand (BOD) determination is an empirical test in which standardization laboratory procedures are used to determine the relative oxygen requirement of waste waters, effluents and polluted waters. The method consists of filling an airtight BOD bottle of a specified size with a buffered test sample (pH 6.5 – 7.5) and incubation for a specified temperature (20 ^{o}C) and time (5 days). The dissolved oxygen is measured initially and after the incubation period using dissolved oxygen electrode, and the BOD is computed from the difference between initial and final DO. The method excludes the use of chemicals and manipulation steps.

Equipment:

- Incubation BOD bottles.
- Air incubator, thermostatically controlled at 20±1^{o}C excludes all light to prevent possibility of photosynthetic production of DO.
- Oxygen-sensitive membrane electrode, polarographic or galvanic, or oxygen-sensitive optical probe with appropriate meter.

Reagents:

1. Phosphate buffer solution. Dissolve 8.5 g KH_2HPO_4, 21.75 g K_2HPO_4, and 33. 4 g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl in about 500 ml distilled water and dilute to one liter. The pH should be 7.2 without further adjustment. Discard reagent (or the following reagent) if there is any sign of biological growth in the stock bottle.
2. Magnesium sulfate solution. Dissolve 22.5 g $MgSO_4.7H_2O$ in distilled water and dilute to 1 liter.
3. Calcium chloride solution. Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 liter.
4. Ferric chloride solution. Dissolve 0.25 g $FeCl_3$.$6H_2O$ in distilled water and dilute to 1 liter.
5. Acid and alkali solutions, 1N, for neutralization of caustic or acidic waste samples. Acid solution is prepared by slowly addition while stirring, 28 ml concentrated sulfuric acid to distilled water and dilution to 1 liter. Alkali solution is prepared by dissolving 40 g sodium hydroxide in distilled water and dilution to 1 liter.
6. Nitrification inhibitor. 2-Chloro-6-(trichloromethyl) pyridine reagent-grade is dried at 130°C for 1h, mixed with 150 mg glucose and 150 mg glutamic acid, dissolved in water and diluted to 1 liter.
7. (N.B. The solution is freshly prepared immediately before use).
8. Glucose–glutamic acid (GGA) solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to

reagent-grade water and dilute to 1 liter. Prepare fresh immediately before use unless solution is maintained in a sterile container. Store all GGA mixtures at ≤ 6°C without freezing unless manufacturer recommendations state otherwise. Commercial preparations may be used but concentrations may vary. Discard solutions if evidence of contamination is indicated (e.g., growth occurs in the stock bottle or GGA test results are consistently low).

Procedure:

1. Preparation of dilution water. Place the desired volume of water in a suitable bottle and add 1.0 ml each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions/l. Before use, bring dilution water to 20°C and saturate with DO by shaking in a partially filed bottle or by aerating with organic-free filtered air.
2. Dilution water check. Incubate a BOD bottle full of dilution water for 5 days at 20 °C. Determine the initial and final DO and calculate the DO uptake. The difference should not be more than 0.2 mg/l and preferably not more than 0.1 mg/l.
3. Glucose- glutamic acid check. Use a mixture of 150 mg glucose/l and 150 mg glutamic acid/l as a standard check solution. Determine the BOD (5 days, 20 °C) of 2 % dilution of the glucose – glutamic acid standard check solution. The accepted control limits are in the range of 198 ±2 mg O_2. If measured BOD for a glucose glutamic acid check is outside the range, reject testes made with that seed and dilution water.
4. Seed control. Determine BOD of the seeding material as for any other samples, the DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/l.
5. Sample pretreatment. For sample containing caustic alkalinity or acidity, the sample is neutralized to pH 6.5 to 7.5.
6. Dilution technique. Dilutions result in a residual DO of at least 2 mg/l after 5 days incubation period. 0.0 to 1 % for strong industrial wastes, 1 to 5% for raw and settled waste water, 5 to 25 % for biologically treated effluent, and 25 to 100 % for polluted river waters.
7. For most routine lagoon samples three dilutions are made, (1:30), (1:10) and (1:3). For lagoon samples with a strong odor or odor of sewage, the sample is diluted 100 times.
8. Industrial samples are diluted by the ratio (1:5), (1:20), (1:100), (1:500), (1: 2000), and tested to determine the approximate BOD.
9. Prepare dilution either in graduated cylinders and then transfer to BOD bottles or prepare directly in BOD bottles.
10. When using graduated cylinders to prepare dilutions, and when seeding is necessarily, add seed either directly to dilution water or to individual cylinders before dilution. When dilutions are prepared directly in BOD bottle and when seeding is necessarily, add seed directly to dilution water or directly to BOD bottles.

11. Dilution prepared in graduated cylinders. Siphon dilution mixture into one BOD bottle. Determine the initial DO in this bottle and replace any displaced content with sample dilution to fill the bottle. Stopper tightly, water–seal, and incubate for 5 days at 20 °C.
12. Dilution prepared directly in BOD bottles. Using a wide tip volumetric pipette, add the desired volume to individual BOD bottles of known capacity, add appropriate amounts of seed material to the individual BOD bottles or to the dilution water. The bottles are filled with enough dilution water, seeded if necessary, so that insertion of stopper will displace all air, leaving no bubbles. For dilution greater than 1:100 make a primary dilution in a graduated cylinder before making the final dilution in the bottle. If the membrane electrode method is used for DO measurement, prepare only one BOD bottle for each dilution. Determine the initial DO in the bottle and replace any displaced contents with dilution water to fill the bottle, stopper tightly, water-seal, and incubate for 5 days at 20°C, rinse DO electrode between determination to prevent cross-contamination of samples.
13. Determination of initial DOES if the sample contains materials that react rapidly with DO, determine the initial DO immediately after filling the BOD bottle with diluted sample. By using membrane-electrode method or optical-probe method.
14. Dilution water blank. Together with each batch of samples incubate a bottle of unseeded dilution water. Determine the initial and final DO, the DO uptake should not be more than 0.2 mg/l.
15. Incubation. Incubate at 20°C the bottles containing desired dilution, seed control, dilution water blank, and glucose- glutamic acid check.
16. Determination of the final DO. After 5 days of incubation, determine DO in the sample dilutions, blanks, and checks. If more than one sample dilution meets the criteria of residual DO of at least 1 mg/ liter and a DO depletion of at least 2 mg/liter and the dilution water blank doesn't deplete by more than 0.5 mg/l, average all results in the acceptance range. If the dilution water blank deplete by more than 0.5 mg/liter use the results of dilution with the highest sample concentration.
17. Quality control. Perform glucose- glutamic acid check.

Calculation:

A. When dilution water is not seeded:

$$BOD_5, (mg/l) = \frac{[(D_1 - D_2)]}{P}$$

B. When dilution water is seeded:

$$BOD_5, (mg/l) = \frac{[(D_1 - D_2) - (B_1 - B_2)]}{P}$$

where:

D_1= DO of diluted sample immediately after preparation, mg/l.

D_2= DO of diluted sample after 5 days incubation at 20 °C, mg/l.

P = Decimal volumetric fraction of sample used .

B_1= DO of seed control before incubation, mg/l.

B_2= DO of seed control after incubation, mg/l.

Precautions:

1. Avoid cross contamination by keeping the sample container closed till using the sample.
2. Incubation of BOD bottles (250 to 300 ml capacity) and keeps sterilized.
3. Clean the BOD bottles with a detergent, rinse thoroughly, and drain before use.
4. Air incubator or water bath thermostatically controlled at 20±1 ^{o}C is commonly used. Exclude all light to prevent possibility of photosynthetic production of DO.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method 5210 (B).

(IV.6) Biochemical Oxygen Demand (5-Day BOD)

(Manometry - Respirometry)

Principle:

The respirometric method provides a direct measurement of the oxygen consumed by microorganisms from an air or oxygen enriched environment in a closed vessel containing the test sample under conditions of constant temperature and agitation. In this method, manometric respirometers are used, and relate the oxygen uptake to the change in pressure caused by oxygen consumption while maintaining a constant volume.

Equipment:

1. DO meter.
2. pH meter.
3. Respirometer system: OxiTop measuring system.
4. Inductive stirring system.
5. Incubator thermostatic box (temperature 20±1°C).
6. Sample bottles, brown (normal volume 510 ml).
7. Stirring rods.
8. Stirring rods remover.
9. Suitable overflow measuring beakers.
10. Rubber quivers.

Reagents:

1. Sodium hydroxide tablets.

2. Distilled water. Use only high quality water distilled from all glass still. Deionized water may be used, but often contains high bacterial counts. The water must contain less than 0.01 mg heavy metals/l and be free of chlorine, chloramines, caustic alkalinity, and organic material or acids.

3. Phosphate buffer solution:

Potassium dihydrogen phosphate (KH_2PO_4) ……..	8.5 g
Dipotassium hydrogen phosphate (K_2HPO_4) ……	21.75 g
Di-sodium hydrogen phosphate-12-hydrate ($Na_2HPO_4.2H_2O$)	44.78 g
Ammonium chloride ……..	1.7 g
Reagent water …….	1000 ml

Preparation: Dissolve the first four chemicals in about 500 ml reagent water and dilute to one liter. The pH of this buffer solution should be 7.2 without the need for any adjustments.

OR; Phosphate buffer solution, 1.5 N: Dissolve 207 g sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) in water. Neutralize to pH 7.2 with 6 N potassium hydroxide (KOH) and dilute to 1 liter.

4. Magnesium sulfate solution 0.41 N:

Magnesium sulfate, hepta-hydrate ($MgSO_4.7H_2O$) 22.5 g

Reagent water…….. 1000 ml

Preparation. Dissolve magnesium sulfate in reagent water and dilute to one Liter (1.0 ml ≡ 10 mg Mg).

5. Calcium chloride solution (0.25N):

Calcium chloride ($CaCl_2$)…… 27.5 g

Reagent water …… …… 1000 ml

Preparation. Dissolve calcium chloride in reagent water and dilute to one Liter (1.0 ml ≡ 10 mg Ca).

6. Ferric chloride solution:

Ferric chloride, hexa-hydrate ($FeCl_3.6H_2O$) …… 4.84 g

Reagent water …… 1000 ml

Preparation. Dissolve ferric chloride in reagent water and dilute to one liter (1.0 ml ≡ 1.0 mg Fe).

7. Dilution water. Add 1 ml of each of saline solution per approximately 500 ml water. Dilute to1000 ml and mix. Aerate in suitable apparatus for at least 8 mg/l dissolved oxygen concentration.H_2SO_4, 1 N. Dissolve 28 ml of concentrated H_2SO_4 in distilled water and dilute to one liter.

8. NaOH, 1 N. Dissolve 40 g NaOH in 500 ml distilled water and dilute to one liter.

9. Sodium sulfite solution, 0.025 N:

Sodium sulfite, (Na_2SO_3)…… 1.575 g

Reagent water ……1000 ml

Preparation. Dissolve sodium sulfite in 800 ml water and dilute to one liter.

10. Nitrification inhibitor: Reagent-grade 2- chloro-6-(trichloro-methyl) pyridine TCMP or equivalent.

11.Seeding and seed source: It is necessary to have present population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater unchlorinated or otherwise undisinfected effluents from biological waste treatment plants and surface waters receiving wastewater discharges contain satisfactory microbial population are used for BOD determination of the heavily organic polluted sewage.

- For untreated industrial wastes, disinfected wastes, high temperature wastes or wastes with extreme pH values you, seeding of the diluted samples by adding a population of microorganisms must be performed.
- The preferred seed is from a biological treatment system processing the waste. Where this is not available, use supernatant from domestic wastewater after settling for at least one hour but no longer than 36 hours.
- Some samples may contain materials not degraded at normal rates by the microorganisms in settled domestic, seed with an adapted microbial population

obtained from the disinfected effluent of biological treatment plant.

- In absence of such a facility, obtain seed from the receiving water below the point of discharge (3-8 Km).
- An adapted seed can be developed in the lab by continuously aerating a sample of settled domestic wastewater and adding small daily increments of waste. Optionally use a soil suspension or activated sludge, or a commercial seed preparation to obtain the initial microbial population.

Procedure:

A. Sample pre-treatment:

Homogenize samples that contain particulate matter such as algae or other suspended matter. Measure the initial DO of the sample saturated with oxygen.

1. Check the pH of each sample and neutralized to pH 6.5 to 8.5.
2. Test samples of extreme pH ranges are neutralized to pH 6.5 and 8.5 with a solution of sulfuric acid or sodium hydroxide of such strength that the quantity of reagent does not dilute the sample by more than 0.5 %. Neutralize enough sample for the analysis. Use the neutralized aliquot for the BOD analysis. The pH of the seeded diluted sample should not be affected by the lowest sample dilution. Samples that are neutralized by this way must be seeded.
3. Temperature adjustment. Bring samples and dilution water to desired test temperature (±1°C) before making dilutions or transferring to test vessels.
4. Samples containing residual chlorine are dechlorinated and diluted by seed dilution water. If the sample has been chlorinated, but residual chlorine is not detected, seed the dilution water. Chlorine may dissipate within 1-2 hour standing in the light. To destroy chlorine, add Na_2SO_3 solution. The required volume is determined by titration.
5. Use 100-1000 ml of neutralized sample, add 10 ml of (1+1) acetic acid or (1+50) H_2SO_4, 10 ml KI solution (10 g/100 ml) per 1000 ml portion, and titrate with Na_2SO_3 solution to the starch -iodine end point.

B. Sample dilution:

1. Measure the COD to have a guide for sample volume and dilution needed.
2. Choose the volume of the sample according to Table (IV. 2).

Table (IV.2) Volumes of the samples and dilution factor

Sample volume, ml	Measring range, mg/l	Factor
432	0 - 40	1
365	0 - 80	2
250	0 - 200	5
164	0 - 400	10
97	0 - 800	20
43.5	0 - 2000	50
22.7	0 - 4000	100

3. Sample dilution is carried out for heavily polluted sewage or industrial water using water of dilution seeded with known volume of source of seed.
4. Rinse measuring bottle with sample, put the magnetic stirring rod into the bottle and insert a rubber quiver in the neck of the bottle.
5. Put 2 sodium hydroxide tablets into the rubber quiver with a tweezers (Caution: the tablets must never come into the sample).
6. Nitrification inhibition: If nitrification inhibition is desired, add 10 mg TCMP/l sample in the test vessel.
7. Seeding: Use enough seed culture to prevent major lags in the oxygen-uptake reaction but not so much that the seed's oxygen uptake exceeds about 10% of the seeded sample's oxygen uptake
8. Screw OxiTop directly on sample bottle (tightly closed). Keep the measuring bottle with OxiTop put on for 5 days at 20±1 ºC in the incubator. After the measuring temperature has been reached (after 1 hour at the earliest, after 3 hour at the latest), the OxiTop automatically starts the measurement of the oxygen consumption.
9. Each day note and record the temperature of the incubator, not necessarily including weekends. During the 5 days, the sample is continuously stirred. The OxiTop automatically stores one value every 24 hours for 5 days. To know the current value press M Key in the system.
10. Readout of the stored values after the 5 days has passed. Dispose of leftover sample and rinse BOD bottle at least twice with tap water. Wash BOD bottles in dishwasher and store in BOD cupboard.

Calculation:

1. Convert the displayed measured value (digits) into the BOD value according to the table illustrated above:

$$\text{Digits x factor} = BOD_5 \text{ in mg/l.}$$

2. Seeded samples:

BOD mg/l = BOD of seeded sample – BOD of the seed

= (digits) x factor for seed sample – (digits) x factor for control seed x V1/V2

where,

V1 = volume (ml) of dilution water including seed in sample.

V2 = volume (ml) of undiluted sample.

Correct oxygen uptake for seed and dilution as follows:

$$C = [A - B\,(SA/SB)]\,(1000/NA)$$

Where:

C = corrected oxygen uptake of sample, mg/l,

A = measured oxygen uptake in seeded sample, mg,

B = measured oxygen uptake in seed control, mg,

SA = volume of seed in sample A, ml,
SB = volume of seed in sample B, ml,
and *NA* = volume of undiluted sample in sample A, ml.

Precautions:

Discard any reagent showing signs of biological growth or chemicals precipitation. Stock solutions can be sterilized by autoclaving to provide longer shelf life. Note that excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramines compounds if present. Sample containing toxic substances often require special study and treatment.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, (Methods 5210B, 5210D).

(IV.7) Oil and Grease, Total

(Gravimetry)

Principle:

The test sample solution is acidified to pH < 2 and serially extracted with cyclohexane in a separatory funnel. The extract is dried over anhydrous sodium sulfate, filtered, evaporated and the residue is weighed. The method covers the range of 5 - 1000 mg/l of extractable materials.

Equipment:

1. Separatory funnel, with Teflon stopcock.
2. Vacuum pump or other source of vacuum.
3. Boiling flask, 125 ml.
4. Distilling head, Claisen or equivalent.
5. Filter paper, Whatman No. 40, 11 cm.

Reagents:

1. Hydrochloric acid, (1:1). Mix equal volumes of concentrated HCl and distilled water.
2. Cyclohexane, or trichlorotrifluoroethane, n-hexane and methyl-tert.-butylether.
3. Sodium sulfate anhydrous crystal.

Procedure:

1. Mark the sample bottle at the water meniscus for later determination of sample volume. If the sample was not acidified at the time of collection, add 5 ml hydrochloric acid (1:1) to the sample bottle. After mixing, check the pH by touching pH-sensitive paper to the cap to insure that the pH is 2 or lower. Add more acid if necessary. Pour the sample into a separatory funnel.

2. Add 30 ml cyclohexane or trichlorotri-fluoroethane or a (1+1) mixture of n-hexanemethyl-tert. -butylether to the sample bottle and rotate the bottle to rinse the sides. Transfer the solvent into the separator funnel. Extract by shaking vigorously for 2 minutes. Allow the layers to separate, and filter the solvent into the separator funnel.

3. Extract by shaking vigorously for 2 minutes. Allow the layers to separate, and filter the solvent layer into the flask through a funnel containing solvent moistened filter paper.

4. Repeat the extraction step twice more, with additional portions of fresh solvent, combine altogether the extract portions. Transfer all solvent extract to a pre-dried boiling flask.

5. Rinse the tip of the separatory funnel, the filter paper and then the funnel with a total of 10 - 20 ml of solvent and collect the rinsing in the flask.

6. Connect the boiling flask to the distilling head and evaporate the solvent by immersing the lower half of the flask in water at 85 °C. Collect the solvent for reuse. A solvent blank should accompany each set of samples.

7. When the temperature in the distilling head reaches 50 °C, or the flask appears dry remove the distilling head. Sweep out the flask for 15 s with air to remove solvent vapor by inserting a glass tube connected to a vacuum source. Remove the flask from the heat source and wipe the outside to remove excess moisture and fingerprints. Cool in a desiccator for 30 min and weigh.

Calculation:

$$\text{Total oil and grease, mg /l} = \frac{(A-B)*1000}{\text{volume of sample}}$$

where:

A = residue, gross weight of extraction flask minus the tare weight, in milligrams.

B = blank determination, residue of equivalent volume of extraction solvent, in milligrams.

V = volume of sample (liter) determined by refilling sample bottle to calibration line and correcting for acid addition, if necessary.

Precautions:

1. An emulsion that fails to dissipate can be broken by pouring about 1 g sodium sulfate into the filter paper cone and slowly draining the emulsion through the salt. Additional 1 g portions can be added to the cone as required.

2. A representative sample of 1.0 liter volume should be collected in a glass bottle. If analysis is to be delayed for more than a few hours, the sample is preserved by the addition of 5 ml HCl (1:1) at the time of collection and refrigerated at 4 °C.

3. Because losses of grease will occur on sampling equipment, the collection of a composite sample is impractical. Individual portions collected at prescribed time intervals must be analyzed separately to obtain the average concentration over an extended period.

4. The method is not applicable to measurement of light hydrocarbons that volatilize at temperatures below the boiling point of the solvent used.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 413.1).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5520-B).

(IV.8) Oil and Grease, Total

(Partition-Infrared Spectrometry)

Principle:

The use of tri-chloro-trifluoro-ethane as an extraction solvent for oil and grease allows sensitive detection of the absorbance of the carbon-hydrogen bond in the infrared region of the spectrum at a peak maximum of 2930 cm^{-1}. This can be used to measure oil and grease. Elimination of the evaporation step permits infrared detection of many relatively volatile hydrocarbons. Thus, the lighter petroleum distillates, with the exception of gasoline, may be measured accurately. With adequate instrumentation, as little as 0.2 mg oil and grease/l can be measured.

Equipment:

1. Separatory funnel, 2-liters.
2. Volumetric flask, 100-ml.
3. Funnel, glass.
4. Filter paper, 11-cm diameter.
5. Centrifuge, capable of spinning at least four 100-ml glass centrifuge tubes at 2400 rpm or more.
6. Centrifuge tubes, 100-ml, glass.
7. Infrared spectrophotometer, double-beam, recording.
8. Cells, near-infrared silica.

Reagents:

1. Hydrochloric acid, HCl (1:1).
2. Trichlorotrifluoroethane (1,1,2-trichloro-1,2,2-trifluoroeth-ane), boiling point 47°C. The pure solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.
3. Sodium sulfate, Na_2SO_4, anhydrous, crystal. Dry at 200 to 250 °C for 24 h.
4. Reference oil: Prepare a mixture, by volume, of 37.5 % isooctane, 37.5 % hexadecane, and 25.0 % benzene. Store in a sealed container to prevent evaporation.

Procedure:

1. After carefully transferring of a known volume of the test sample to a separatory funnel, rinse the sample bottle with 30 ml trichlorotrifluoroethane and add solvent washings to the funnel. Shake vigorously for 2 min.

2. Let layers separate. Drain all but a very small portion of the lower trichlorotrifluoroethane layer through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, 100-ml volumetric flask.

3. If a clear solvent layer cannot be obtained and an emulsion of more than about 5 ml exists, extract twice more with 30 ml solvent each time, but first rinse sample container with each solvent portion.

4. Repeat centrifugation step if emulsion persists in subsequent extraction steps.
5. Combine all the extracts including the final rinsing of the filter paper and Na_2SO_4 with an additional 10 to 20 ml solvent and transfer to the volumetric flask. Adjust final volume to 100 ml with the solvent.
6. Prepare a stock solution of known oil by rapidly transferring about 1.0 ml (0.5 to 1.0 g) of the oil or grease to a tared 100-ml volumetric flask. Stopper flask and weigh to nearest milligram.
7. Add solvent to dissolve the sample and dilute to mark. If the oil identity is unknown, use the reference oil as the standard.
8. Using volumetric techniques, prepare a series of standards over the range of interest. Select a pair of matched near-infrared silica cells.
9. A 1.0-cm path-length cell is appropriate for a working range of about 4 to 40 mg.
10. Scan standards and samples from 3200 cm^{-1} to 2700 cm^{-1} with the solvent blank in the reference beam and record results on absorbance paper.
11. Measure absorbance of samples and standards by constructing a straight base line over the scan range and measuring absorbance of the peak maximum at 2930 cm^{-1} and subtracting baseline absorbance at that point. If the absorbance exceeds 0.8 for a sample, select a shorter path length cell or dilute the sample as required.
12. Use scans of standards to prepare a calibration curve.

Calculation:

$$\text{mg oil and grease/l} = A \times 1000 / \text{ml sample}$$

where: A = mg of oil or grease in the extract as determined from calibration curve.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5520 (C).

(VI.9) Total Organic Nitrogen

(Potentiometry - Kjeldahl Method)

Principle:

The amino nitrogen of many organic materials is converted into ammonium sulfate [$(NH_4)_2SO_4$] upon digestion with H_2SO_4, and a mixture of potassium sulfate (K_2SO_4), and mercuric sulfate ($HgSO_4$) as a catalyst. Free ammonia and ammonium-nitrogen are also converted into ammonium sulfate. During sample digestion, a mercury ammonium complex is formed, which subsequently decomposed into ammonium ion by sodium thiosulfate ($Na_2S_2O_3$). After decomposition, ammonia is distilled from an alkaline medium and absorbed in boric or sulfuric acid. The ammonia is measured potentiometrically, colorimetrically or visual titrimetrically. The potentiometric method covers the range 0.05 - 1400 mg nitrogen/l.

Equipment:

1. Digestion apparatus: Kjeldahl flasks of 800 ml capacity are generally used.
2. A heating device for the Kjeldahl flasks, to provide a temperature range of 365 to 370 °C.
3. Distillation apparatus.

Reagents:

All the reagents listed for the determination of ammonia-nitrogen are required in addition to the following:

1. Mercuric sulfate solution: Dissolve 8 g of red mercuric oxide, HgO, in 100 ml of 6 N H_2SO_4.
2. Digestion reagent. Dissolve 134 g of K_2SO_4 in 650 ml of ammonium free water. Add 200 ml of concentrated H_2SO_4. While stirring, add 25 ml of mercuric sulfate solution to 1 liter with ammonium free water. Keep at a temperature close to 20°C to prevent crystallization.
3. Sodium hydroxide - sodium thiosulfate reagent. Dissolve 500 g of NaOH and 25 g of $Na_2S_2O_3.5H_2O$ in ammonia free water and dilute to one liter.
4. Borate buffer solution.
5. Sodium hydroxide solution: NaOH, 6 N.

Procedure:

1. Selection of sample size and preparation of sample. Transfer a measured volume of the test sample (25 - 500 ml) or a quantity equivalent to 500 - 2500 µg/ml organic nitrogen to 800 ml Kjeldahl flask. If necessary, dilute the sample and neutralize to pH 7.

2. Ammonia removal. Add 25 ml borate buffer and then 6 N NaOH until pH 9.5 is reached. Add a few glass beads and boil for 5 minutes. If desired, distill this fraction

and determine the ammonia nitrogen. Alternatively, if ammonia has been determined by the distillation method, use the residue in the distillation flask for the organic nitrogen determination.

3. Digestion. Cool, and carefully add 50 ml of the digestion reagent to the Kjeldahl flask. Add a few glass beads and, after mixing, heat under a hood with exhaust facility to remove acid fumes. Boil till the volume is reduced to 20 - 50 ml and white fumes are observed. As digestion continues, colored or turbid samples will turn clear or straw colored. After digestion let the flask and content cool, and dilute to 300 ml with ammonia free water and mix. Tilt the flask and carefully add 50 ml of hydroxide-thiosulfate reagent to form an alkaline layer at the bottom of the flask. Shake the flask, a black precipitate of HgS will form and the pH should exceed 11.0. Connect the flask to the cleaned distillation apparatus.
4. Collection of ammonia. Distill and collect 200 ml distillate below the surface of 50 ml absorbant solution. Use plain boric acid solution when ammonia is to be determined by Nesslerization, or use indicating boric acid when the titrimetric method will be used for analysis.
5. Final ammonia measurement. The method of ammonia measurement by Nesslerization and titrimetric analysis is explained in the ammonia section.
6. Blank. Carry out a reagent blank through all steps of the procedure and apply the necessary corrections to the results.

Calculation:

Same as for ammonia (Section II. 10).

Precautions:

The most reliable results are obtained on fresh samples. If it is not possible to analyze immediately, acidify the sample to pH = 1.5 to 2.0 with H_2SO_4 and store at 4°C.

Interferences:

1. During Kjeldahl digestion, nitrate in excess of 10 mg/l can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference in conjunction with the Kjeldahl methods described herein.
2. Inorganic salts and solids: The acid and salt content of the Kjeldahl digestion reagent is intended to produce a digestion temperature of about 380 °C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400 °C, at which point pyrolytic loss of nitrogen begins to occur.

3. To prevent an excessive digestion temperature, add more H_2SO_4 to maintain the acid-salt balance. Not all salts cause precisely the same temperature rise, but adding 1 ml H_2SO_4/g salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 351.4).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5520 (C).

(IV.10) Phenols, Total Recoverable

(Spectrophotometry - Aminoantipyrine Method)

Principle:

Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at pH 10 to form a stable reddish-brown colored antipyrine dye. The intensity of the color produced is a function of the concentration of phenolic materials. The method is sensitive enough to measure as low as 50 μg/l phenolic compounds in aqueous solutions. For determining total recoverable phenol, a steam distillation step is required, and the reaction is performed on the distillate.

Equipment:

Distillation apparatus, all glass consisting of a 1 liter boro-silicateglass distilling apparatus with Graham condenser.

1. pH meter.
2. Spectrophotometer, for use at 460 or 510 nm.
3. Funnels.
4. Filter paper.
5. Membrane filters.
6. Separatory funnels, 500 or 1000 ml.
7. Nessler tubes, short or long form, cuvettes 1.0 cm.

Reagents:

1. Phosphoric acid solution, (1+9): Dilute 10 ml of 85% H_3PO_4 to 100 ml with distilled water.

2. Copper sulfate solution. Dissolve 100 g $CuSO_4.5H_2O$ in distilled water and dilute to 1 liter.

3. Buffer solution. Dissolve 16.9 g NH_4Cl in 143 ml concentrate aqueous ammonia and dilute to 250 ml with distilled water. Addition of 2 ml should adjust 100 ml of the distillate to pH 10.

4. 4-Aminoantipyrine (AAP) solution. Dissolve 2 g of 4-AAP in distilled water and dilute to 100 ml.

5. Potassium cyanoferrate (III) solution. Dissolve 8 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 ml.

6. Stock phenol solution. Dissolve 1.0 g phenol in freshly boiled and cooled distilled water and dilute to 1 liter (1.0 ml ≡ 1.0 mg phenol).

7. Working solution (A). Dilute 10 ml stock phenol solution to 1 liter with distilled water (1.0 ml ≡ 10.0 μg phenol).

8. Working solution (B). Dilute 100 ml of working solution (A) to 1000 ml with distilled water (1.0 ml ≡ 1.0 μg phenol).

9. Chloroform.

10. Ferrous ammonium sulfate. Dissolve 1.1 g ferrous ammonium sulfate in 500 ml distilled water containing 1 ml of concentrated H_2SO_4 and dilute to 1 liter with freshly boiled and cooled distilled water.

Procedure:

A. Distillation:

1. Transfer 500 ml of the test sample into a beaker. Adjust the pH to approximately 4 with (1+9) H_3PO_4, add 5 ml $CuSO_4$ solution and transfer to the distillation apparatus. Omit adding H_3PO_4 and $CuSO_4$ if the sample was preserved.

2. Distill 450 ml of the sample, stop the distillation, and when boiling ceases add 50 ml of warm distilled water to the flask and resume distillation until 500 ml have been collected.

3. If the distillate is turbid, filter through a prewashed membrane filter.

B. Direct photometric measurement:

1. Using the working solution (A), transfer 0 - 8 ml of the standard (0 - 800 μg/l phenol) to 100 ml volumetric flasks and dilute to the mark with water.

2. To 100 ml of sample distillate or an aliquot diluted to 100 ml and/or standards, add 2 ml of the buffer solution and mix. The pH of the sample and standards should be 10 ± 0.2.

3. Add 2.0 ml 4-aminoantipyrine solution and mix.

4. Add 2.0 ml potassium cyanoferrate (III) solution and mix.

5. After 15 minutes read absorbance at 510 nm.

C. Chloroform extraction method:

1. Using the working solution (B), transfer 0 - 25 ml of standard (0 - 50 μg/l) phenol to the separatory funnels and dilute to 500 ml with distilled water.

2. Place 500 ml of the distillate or an aliquot diluted to 500 ml in a separatory funnel. The sample should not contain more than 25 μg phenol.

3. To the test sample and standards add 10 ml of the buffer solution and mix. The pH should be 10 ± 0.2.

4. Add 3.0 ml 4-aminoantipyrine solution and mix.

5. Add 3.0 ml potassium cyanoferrate (III) solution and mix.

6. After 3 minutes, extract with 25 ml chloroform. Shake the separatory funnel for at least 10 times, let $CHCl_3$ settle, shake again 10 times and let chloroform settle again. Vent chloroform fumes into hood.

7. Filter chloroform extracts through filter paper. Do not add more chloroform.

8. Carry out filtration in a hood.

9. Dispose chloroform in environmentall acceptable manner.

10. Read the absorbance of the samples and standards against the blank at 460 nm.

Calculation:

1. Prepare a calibration curve by plotting the absorbance value of the standards versus the corresponding phenol concentrations.
2. Obtain concentration value of the unknown test sample directly from the calibration curve.

$$\text{Phenols, (mg/l)} = A/B \times 1000$$

Where:

A = phenol content (μg) in sample from calibration graph.

B = volume (ml) of original sample.

Precautions:

1. Biological degradation is inhibited by the addition of 1 g/l of copper sulfate to the test sample and acidification to a pH of less than 4 with phosphoric acid. The sample should be kept at 4°C and analyzed within 24 hours after collection.
2. Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of less than 4 with H_3PO_4 and aerating briefly by stirring and adding $CuSO_4$.
3. Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate. If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 420.1).
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5530 C).

(IV.11) Determination of Individual Phenolic Compounds

(Liquid–Liquid Extraction- Gas Chromatographic Method)

Principle:

This method covers the determination of phenol and other 10 phenolic compounds using (GC-FID) technique. It is applicable to municipal and industrial discharges samples. Approximately 1 liter volume of the test sample is acidified and extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated by exchange with isopropanol to a volume of 10 ml or less. An aliquot of the extract is injected in a gas chromatograph equipment, equipped with a flame ionization detector. Standards of phenols are dosed into organic free water and the samples are extracted and analyzed in an identical manner in order to compensate for possible extraction losses.

Table (IV.3) Chromatographic conditions and method detection limit

Compound	Retention time, min	Method detection limit, µg/l
2-Chlorophenol	1.70	0.31
2,4-Dimethylphenol	4.03	0.32
2-Methyl-4,6-dinitrophenol	10.24	16
4-Nitrophenol	24.25	2.8
Phenol	3.01	0.14
4-Chloro-3-methylphenol	7.50	13.0
2,4-Dichlorophenol	4.3	0.39
2,4-Dinitrophenol	10.00	13.0
2-Nitrophenol	2.00	0.45
Pentachlorophenol	12.42	7.4
2,4,6-Trichlorophenol	6.05	0.64

Equipment:

1. Micro syringes of volumes, 10 µl, 25 µl, 50 µl, 100 µl, 250 µl and 1000 µl.
2. Separatory funnel, 2liter capacity, with Teflon stopcock.
3. Volumetric flasks, 5 ml, 10 ml with glass stopper.
4. Erlenmeyer flask, 500 ml.
5. Evaporative flask, Kuderna-Danish (KD), 500 ml, or Rotary evaporator.
6. Receiving tube, 10 ml.
7. Snyder column, 3-ball design, threaded joint.
8. Graduated cylinder, 1000, and 100 ml.
9. Boiling chips, heated to 44 oC for 30 minutes, cooled, and stored in a desiccator before use.
10. Glass Pasteur pipettes.
11. Autosampler vials 2 ml with crimp tops.

12. Water bath, heated, with concentric ring cover.

13. 2-Ball micro Snyder column.

14. Dispenser, bottle-top. 2-10 ml capacity micro syringes, 10 ul, 25 µl, 100 µl ,250 µl, 500 µl and1000 µl.

15. Standard solution storage containers - 5 ml vial round, amber glass bottles with TFE-lined caps.

16. Gas Chromatograph system, equipped with split / splitless injector with single tapered liner and a flame ionization detector, with auto- sampler.

17. Capillary chromatographic column CP Sill-8 (50 m x 0.25 mm, bonded fused silica column, 0.25 µm) or equivalent, and software for acquisition and data analysis.

18. Analytical balance, with an accurately of ± 0.0001 g.

Reagents

1. Reagent or pesticide grade chemicals shall be used.

2. Sodium sulfate, anhydrous, baked at 400 0C for four hours.

3. Sulfuric acid, 50 % (v/v) in organic free water.

4. Sodium hydroxide, 10 N. prepared in organic free water.

5. Isopropanol.

6. Methylene chloride.

7. De-ionized water.

8. Sodium thiosulphate - high purity grade.

9. Stock standard phenols solution, 2000 µg/ml.

10. Intermediate standards of concentration of 100 µg/ml (should be stored at 4 0C).

11. Calibration standards: are prepared at five levels as follow:

Table (IV.4) Calibration standards

Std level	Intermediate volume taken (µl)	Final volume (ml)	Final conc. (µg/ml)	Final conc. (µg/l)
Level 1	25	5	0.5	0.5
Level 2	50	5	1	1
Level 3	50	1	5	5
Level 4	100	1	10	10
Level 5	200	1	20	20

1. The levels of intermediate and calibration standards may vary according to the concentration of the Phenols in the purchased stock solutions, all solutions are to be stored at 4 0C.

2. Laboratory fortified blank (LFB) spiking mixture is prepared in methanol using stock standard chlorinated phenols.
3. Working LFB spike solution isprepared at concentration of 8 µg/ml. This solution should be stored at 4 0C.

Procedure:

Sample extraction

1. Prepare one LFB every 20 samples or each batch of samples, then add 1.0 ml of the solution in 1 l reagent water then extract at the same time as the samples.
2. Prepare a method blank every 20 samples or each batch of samples; Then extract at the same time as the samples.
3. Use 1000 ml graduated cylinder to measure and transfer 1 liter of samples to a 2-liter separatory funnel.
4. Adjust the samples to a pH of 1 to 2 with sulfuric acid. Add 60 ml of methylene chloride to the empty samples bottle, cap, and swirl to rinse the inner surfaces.
5. Transfer the solvent to the separatory funnel and extract by shaking the funnel for 2 minutes, with periodic venting to release pressure.
6. Allow the layers to separate for at least 10 minutes. If an emulsion greater than 1/3 the volume of the solvent layer forms, centrifuge or stir to break up the emulsion.
7. Drain the methylene chloride into a 500 ml Erlenmeyer flask and repeat the extraction procedure for a second, third and fourth time. Combine the extracts in the Erlenmeyer flask
8. Add about 20 g of anhydrous sodium sulfate to the extract in the flask, swirl and cover with aluminum foil. Allow to sit for at least 20 min.
9. Concentrate the extract using a rotary evaporator at 43±2 ^{0}C, without vacuum till 2 ml.
10. Add 4 ml of isopropanol to the extract. Then repeat the concentration using the rotary evaporator at 68±2 ^{0}C, under vacuum till 0.5 ml. (Note: Do not allow the extract to go dry).
11. Collect the extract by syringe then complete to 1.0 ml with isopropanol.

Gas chromatography conditions:
With flame ionization detector:

1. Carrier gas flow: Helium 1.0 ml / min.
2. Nitrogen makeup gas flow: 30 ml / min. H_2 flow: 25 ml / min, air flow: 300 ml / min .
3. Injection: split/ split less.
4. Injection volume: 3 µl.
5. Injector temp.: 280 ºC.
6. Detector temp.: 300 ºC.
7. Thermal program: 70 ºC hold for 2 min, ramp 7 ºC / min. to 240 ºC and hold for 2 min.

Calculation:

1. Identify the samples peak by comparison of its retention time to the retention time of the calibration standards. The retention time of the unknown should fall within the average RT of the standards ± 3 standard deviations. However, the experience of the analyst weighs heavily in the interpretation of the chromatogram.
2. Calculate the corrected concentrations of each analyte in the sample from the response factors of the calibration curves generated.
3. Confirmation is required on GC/MS/MS when exceeding the maximum permissible level (MPL) is suspected.
4. If the response for a peak exceeds the highest standard by more than 20%, dilute the sample and rerun.

Precautions:

1. Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware that lead to discrete materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks.
2. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use, by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water.
3. The glassware should then be drained dry and heated in a muffle furnace at 400 oC for 15 to 30 min. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric glassware should not be heated in the furnace. After dry and cooling, glassware should be sealed and stored in a clean environment or capped.

References

1. "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 6420B).
2. USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 604a).

(IV. 12) Anionic Surfactants, (MBAS)

(Spectrophotometry - Methylene Blue Method)

Principle:

Methylene blue active substance (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by MBAS anion and methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many substances, natural and synthetic, showing methylene blue activity. The MBAS method is useful for estimating the anionic surfactant content of waters and wastewaters. The minimum detectable limit is 10 μg MBAS.

Equipment:

1. Spectrophotmeter, for use at 652 nm, providing a light path of 1.0 cm or longer, or filter photometer providing a light path of 1.0 cm or longer and equipped with a red color filter exhibiting maximum transmittance near 652 nm.
2. Separatory funnels 500 ml with inert Teflon stopcocks and stoppers.

Reagents:

1. Stock LAS solution, weigh an amount of the reference material equal to 1.0 g LAS on a 100 % active basis. Dissolve in water and dilute to 1000 ml (1.00 ml ≡ 1.00 mg LAS). Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly.
2. Standard LAS solution. Dilute 10.0 ml stock LAS solution to 1000 ml with water (1.00 ml ≡ 10.0 μg LAS). Prepare daily.
3. Phenolphthalein indicator solution, alcoholic.
4. Sodium hydroxide, NaOH, 1N.
5. Sulfuric acid, H_2SO_4, 1 N and 6 N.
6. Chloroform, $CHCl_3$. (Caution: Chloroform is toxic and a suspected carcinogen). Take appropriate precautions against inhalation and skin exposure.
7. Methylene blue reagent. Dissolve 100 mg methylene blue in 100 ml water. Transfer 30 ml to a 1000 ml flask. Add 500 ml water, 41 ml 6 N H_2SO_4, and 50 g sodium phosphate monobasic, monohydrate, $NaH_2PO_4.H_2O$. Shake until dissolved. Dilute to 1000 ml.
8. Wash solution. Add 41 ml 6N H_2SO_4 to 500 ml water in a 1000 ml flask. Add 50 g $NaH_2PO_4.H_2O$ and shake until dissolved. Dilute to 1000 ml.
9. Methanol, CH_3OH. (Caution: Methanol vapors are flammable and toxic; take appropriate precautions).
10. Hydrogen peroxide, H_2O_2, 30%.

11. Glass wool. pre-extract with $CHCl_3$ to remove interferences.
12. Water, reagent-grade, MBAS-free. Use for making all reagents and dilutions.

Procedure:

A. Preparation of calibration curve:

Prepare a series of 10 separatory funnels with 0.00, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, 13.00, 15.00 and 20.00 ml standard LAS solution. Add sufficient water to make the total volume 100 ml in each separatory funnel.

B. Ion pairing and extraction:

1. Transfer the test sample to a separatory funnel. Make the solution alkaline by drop-wise addition of 1 N NaOH, using phenolphthalein indicator. Discharge pink color by dropwise addition of 1 N H_2SO_4. Add 10 ml $CHCl_3$ and 25 ml methylene blue reagent.

2. Rock funnel vigorously for 30 s and let phases separate. Alternatively, place a magnetic stirring bar in the separatory funnel; lay funnel on its side on a magnetic mixer and adjust speed of stirring to produce a rocking motion. Excessive agitation may cause emulsion formation. To break persistent emulsions, add a small volume of isopropyl alcohol (< 10 ml); add same volume of isopropyl alcohol to all standards. Some samples require a longer period of phase separation than others.

3. Before draining $CHCl_3$ layer, swirl gently, then let settle. Draw off $CHCl_3$ layer into a second separatory funnel. Rinse delivery tube of first separatory funnel with a small amount of $CHCl_3$. Repeat extraction two additional times, using 10 ml $CHCl_3$ each time. If blue color in water phase becomes faint or disappears, discard and repeat, using a smaller sample.

4. Combine all $CHCl_3$ extracts in the second separatory funnel. Add 50 ml washed solution and shake vigorously for 30 s. Emulsions are not formed at this stage. Let settle, swirl, and draw off $CHCl_3$ layer through a funnel containing a plug of glass wool into a 100 ml volumetric flask; filtrate must be clear. Extract wash solution twice with 10 ml $CHCl_3$ each, and add to flask through the glass wool. Rinse glass wool and funnel with $CHCl_3$. Collect washings in a volumetric flask, dilute to mark with $CHCl_3$, and mix well.

C. Measurements:

Determine absorbance at 652 nm against a blank of $CHCl_3$.

Calculation:

From the calibration curve, read micrograms of apparent LAS (molecular weight) corresponding to the measured absorbance.

$$MBAS,(mg/l) = \frac{\text{mg apparent LAS}}{\text{ml original sample}}$$

Report as "MBAS, calculated as LAS, molecular weight....."

Precautions:

1. To avoid decolorization of methylene blue by sulfides, add a few drops of 30% H_2O_2.
2. Most non surfactant aqueous-phase interferences can be removed by sublation.

References:

- USEPA "Methods for Chemical Analysis of Water and Wastes", Ohio, 1983 (Method 425.1).

"Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 [Method 5540(C)].

(IV.13) Total Organic Carbon (TOC)

(Infrared Spectrometry)

Principle:

A micro portion of the homogenized test sample is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide in the TOC equipment. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is transported by the carrier gas stream and is measured by means of a non-dispersive infrared analyzer. Because total carbon is measured, inorganic carbon must be measured separately and TOC obtained by difference.

Equipment:

1. Total organic carbon analyzer.
2. Syringes. 0 - 50 µl, 0 - 200 µl, and 0 - 1 ml capacity.
3. Sample blender or homogenizer.
4. Magnetic stirrer and Teflon-coated stirring bars.
5. Filtering apparatus and 0.45 µm-pore-diameter filters.

Reagents:

1. Reagent water: Prepare blanks and standard solutions from carbon-free water; preferably use carbon-filtered, redistilled water.
2. Phosphoric acid, H_3PO_4, concentrated. Alternatively use sulfuric acid H_2SO_4, but not hydrochloric acid.
3. Organic carbon stock solution. Dissolve 2.1254 g anhydrous potassium biphthalate, $C_8H_5KO_4$, in carbon-free water and dilute to 1000 ml; 1.0 ml ≡ 1.00 mg carbon. Alternatively, use any other organic-carbon-containing compound of adequate purity, stability, and water solubility. Preserve by acidifying with H_3PO_4 or H_2SO_4 to pH < or = 2.
4. Inorganic carbon stock solution. Dissolve 4.4122 g anhydrous sodium carbonate, Na_2CO_3, in water, add 3.497 g anhydrous sodium bicarbonate, $NaHCO_3$, and dilute to 1000 ml (1.00 ml ≡ 1.00 mg carbon). Alternatively, use any other inorganic carbonate compound of adequate purity, stability, and water solubility. Keep tightly stoppered. Do not acidify.
5. Carrier gas. Purified oxygen or air, CO_2-free and containing less than 1 ppm hydrocarbon (as methane).
6. Purging gas. Any gas free of CO_2 and by hydrocarbons.

Procedure:

1. Instrument operation. Follow manufacturer's instructions for analyzer assembly, testing, calibration, and operation. Adjust to optimum combustion temperature (typically 900°C) before using instrument; monitor temperature to insure stability.
2. Sample treatment. If the test sample contains gross solids or insoluble matter, homogenize until satisfactory replication is obtained. Analyze a homogenizing blank consisting of reagent water carried through the homogenizing treatment. Filter sample through 0.45-µm pore-diameter. Filter with vacuum; analyze a filtering blank.
3. Sample injection. Withdraw a portion of prepared sample using a syringe fitted with a blunt tipped needle. Select sample volume according to manufacturer's direction. Stir samples containing particulates with a magnetic stirrer. Select needle size consistent with sample particulate size. Inject samples and standards into analyzer according to manufacturer's directions and record response. Repeat sample injections until consecutive peaks are obtained that are reproducible to within ± 10 %.
4. Preparation of standard curve. Prepare standard organic and inorganic carbon series by diluting stock solutions to cover the expected range in the test samples. Inject and record peak height or area of these standards and a dilution water blank. Plot carbon concentration in milligrams per liter against corrected peak height or area on rectangular coordinate paper. This is unnecessary for instruments provided with a digital readout of concentration. If desirable, prepare a standard curve having concentrations of 1.0 to 10.0 mg/l by making appropriate dilutions of the standards. Inject samples and procedural blanks (consisting of reagent water taken through any pre-analysis steps, values are typically higher than those for reagent water) and determine sample organic carbon concentrations by comparing corrected peak heights to the calibration curve.

Calculation:

1. Calculate corrected peak height of standards by subtracting the reagent-water blank peak height from the standard peak heights.
2. Prepare a standard curve of corrected peak height vs. TOC concentration.
3. Subtract the procedural blank from each sample height and compare to the standard curve to determine carbon content.
4. Apply the appropriate dilution factor when necessary.
5. Subtract the inorganic carbon from the total carbon when TOC is determined by difference.

Precautions:

1. Reagent water is the major contributor to the blank value.
2. Acidification with phosphoric or sulfuric acid to a pH < or = 2 at the time of collection is especially desirable for unstable samples, and may be used on all samples. Acid

preservation, however, requires that inorganic carbon subsequently is purged before analysis.

3. Preserve samples that cannot be examined immediately by holding at 4 °C with minimal exposure to light and atmosphere.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5310 B).

(V) Persistent Organic Pollutants (POP's)

(V.1) Organo-Chlorine Pesticides

(Gas Chromatography - Electron Capture Detector)

Principle:

A measured volume of the test water sample of approximately 1 liter is solvent extracted with methylene chloride by shaking in a separatory funnel. The methylene chloride extract is isolated, dried and concentrated to a volume of 5 ml after solvent substitution with n-hexane. The analytes in the extract are measured by capillary column gas chromatography (GC) equipped with an electron capture detector (ECD). The method is applicable to the chlorinated pesticides shown in Table (V.1).

Table (V.1) Chlorinated pesticides analyzed by gas chromatography-ECD

No.	Analyte	No.	Analyte
1	Aldrin	16	Endrin Aldehyde
2	Atrazine	17	Etridiazole
3	Chlordane-alpha	18	α -HCH
4	Chlordane-gamma	19	β -HCH
5	Chlorneb	20	δ-HCH
6	Chlorobenzilatea	21	ɣ-HCH (Lindane)
7	Chlorothalonil	22	Heptachlor
8	DCPA	23	Heptachlor Epoxide
9	4,4-DDD	24	Hexachlorobenzene
10	4,4-DDE	25	Methoxychlor
11	4,4-'DDT	26	cis-Permethrin
12	Endosulfan I	27	trans-Permethrin
13	Endosulfan II	28	Propachlor
14	Endosulfan Sulfate	29	Trifuluralin
15	Endrin		

Definitions:

1. Internal standard (IS). A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.
2. Surrogate analyte (SA). A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.
3. Laboratory duplicates (D1 & D2). Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of duplicates

give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

4. Laboratory reagent blank (B). An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The blank is prepared if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
5. Laboratory fortified blank (LFB). An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.
6. Stock standard solution. A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.
7. Primary dilution standard solution (PDS). A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.
8. Calibration standard (CAL). A solution prepared from the primary diluted standard solution and stock standard solutions of the internal standards and surrogate analytes. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.

Equipment:

1. Gas chromatograph equipped with a split/splitless injector with single tapered liner, electron capture detector (ECD), and an auto- sampler. Chromatographic column (CP Sill-8, 30 m x 0.25 mm, bonded fused silica 0.25 μm) or equivalent and software for acquisition and data analysis.
2. Rota-vapor with vacuum.
3. pH-meter equipped with glass/reference electrode system for pH measurements. Rinse the electrode(s) with bi-distilled water and dry by gentile wiping with a soft tissue paper. This should be done each time before applying the electrode to a new buffer solution or sample.
4. Borosilicate amber sample bottle (1 liter) with screw caps, lined with Teflon.
5. Separatory funnel (2 liters) with Teflon stopcock, ground glass or Teflon stopper.
6. Micro syringes of volumes, 10, 25, 50, 100, 250 and 1000 µl.
7. Measuring flasks of volumes, 1, 5, 10, 100, and 1000 ml.
8. Amber glass bottles (40 and 20 ml) with Teflon septa screw cap.
9. Disposable (1-2 ml) glass transfer pipettes (Pasteur).

10. Flasks 250 and 500 ml.
11. Auto sampler vials, with crimp caps or screw cap vials with Teflon.
12. Receiving vessels 25, 10, and 4 ml.
13. Balance, analytical, capable of accurately weighing to the nearest 0.0001 g.
14. Cylinder, graduated, 10, 50, 500, and 1000 ml.
15. Standard solution storage containers, 4 ml vial round, amber glass bottles with TFE-lined caps.

Reagents:

1. n-Hexane, with a minimum purity of 95 % and free from pesticides.
2. Acetone, reagent grade.
3. Reagent water.
4. Sodium thiosulfate of the high purity grade.
5. Chlorinated pesticides calibration mixture containing the listed analytes.
6. Working standard solution range (2 µg/ml and 20 µg/ml).
7. Calibration standards (0.01, 0. 02, 0.03, 0.04 and 0.05 µg/l) are prepared from the working standard solution.
8. Pentachloro nitro-benzene (PCNB), 98 % purity, for use as internal standard.
9. Decachlorobiphenyl (DCB), 96 % purity, for use as surrogate standard.
10. Lab fortified blank (LFB) of concentration 0.02 µg/l.
11. Methylene chloride, HPLC grade (for pesticides analysis).
12. Sodium sulfate anhydrous in brown glass bottle (Heat treat in a shallow tray at 400°C for a minimum of four hours to remove interfering organic substances. Store in a glass bottle.
13. Sodium chloride, crystal, ACS grade. Heat treat in a shallow tray at 400 °C for a minimum of four hours to remove interfering organic substances. Store in a glass bottle.
14. Phosphate buffer of pH 7, prepared by mixing 29.6 ml 0.1 N HCl and 50 ml of 0.1 M dipotassium phosphate.
15. Instrument performance check solution prepared from 50 µg/l chlorothalonil, 50 µg/l dimethyl tetra chloroterephthalate (DCPA) and 40 µg/l δ-BHC.
16. GC Degradation check solution prepared from endrin and 4, 4'-DDT each at a concentration of 1 µg/ml.

Procedure:

1. Glassware washing. Wash all glassware used in pesticides analysis by rinsing with hot tap water then with detergent free of carbon and phosphorus then with hot tap water and finally with reagent water. Allow the bottles to dry and seals to air dry, then place in a 400 °C oven for 1 hour or wash with acetone and then allow cooling in cold air. Before starting extraction, wash the glasses with methylene chloride and wash measuring flasks with acetone then with hexane.

2. Sample stabilization. If residual chlorine is present in the test sample, add 80 mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.
3. Sample collection and preservation. Collect the samples with at least one sample in duplicate per batch (batch = 20 samples). Refrigerated sample extracts are stable for 14 days.
4. Extraction. Adjust the test sample solution to pH 7 by adding 50 ml of phosphate buffer and check the pH, add H_2SO_4 or NaOH if necessary. Add 100 g NaCl to the sample, seal, and shake to dissolve salt. Add 60 ml methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to a separatory funnel and extract the sample by vigorously shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 500 ml conical flask. Add a second 60 ml volume of methylene chloride to the sample bottle and repeat the extraction procedure twice, combined the extracts in an Erlenmeyer flask. Perform a third extraction in the same manner. Add about 5 g anhydrous sodium sulfate to dry the extract. Stir the flask to dry extract and allow sitting for 15 minutes. Decant the methylene chloride extract into the Rota vapor flask. Rinse the remaining sodium sulfate with two 25 ml portions of methylene chloride and decant the rinses into the Rota vapor flask till the volume of extract reach to about 5 ml and add 30 ml of hexane. Concentrate the volume to 1.0 ml using Rota vapor under vacuum.
5. Determination. Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest. The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. Helium carrier gas flow is established at 30 cm/sec linear velocity and oven temperature is programmed from 60-300 °C at 4 °C/min. The injector temperature is °C and the detector temperature is 320 °C. Inject using GC/ECD 1 µl extracted sample volume from the concentrate with a 45 second delay.

Calculation:

1. Locate each chlorinated pesticides in the sample chromatogram by comparing the retention time of the suspect peak to the data gathered in method performance data.
2. The retention time of the suspect peak must fall within the limits established during method verification studies for single column identification.
3. Calculate the corrected concentrations of each analyte in the sample from the response factors of the calibration curves generated.

µg analyte/l sample = (A)(B)(D)(E)/(C)(F)

where:

A = sample instrument response.
B = µg analyte/ml in the calibration standard.
C = calibration standard instrument response.
D = ml of the final extract (almost 1.0 ml).
E = dilution factor.
F = amount of samples analyzed in liters for aqueous samples.

Precaution:
Ensure that the samples are preserved in a refrigerator at 4 ^{o}C.

Quality control:

1. Calibration curves are checked by (0.02 and 0.2 µg/l) every analytical batch for each chlorinated pesticides over the concentration range (0.01 - 0.05 µg/l) and (0.1 - 0.5 µg) to confirm the verification of the linear response in these ranges of the system (minimum correlation coefficient is 0.99) and to correct the concentration of each individual chlorinated pesticide.
2. A reagent blank (B) is analyzed at the beginning of each analytical batch. The level of each chlorinated pesticides in the blank reagent has to be less than the LOQ of those chlorinated pesticides.
3. Laboratory duplicates (D1 & D2) are analyzed within each analytical batch. In case replicate sample less than LOQ, no need to measure duplicate sample.
4. Laboratory fortified blank (LFB) is analyzed within each analytical batch and the obtained recovery % shall be within range 70 - 130 %.
5. Degradation of DDT and endrin caused by active sites in the injection port needs a check on the degradation in every injection batch on the GC. Look for the degradation products of 4,4'-DDT (4,4'-DDE and 4,4'-DDD) and the degradation products of endrin (endrin aldehyde, EA and endrin ketone, EK). For 4,4'-DDT, these products will elute just before the parent compound, and for endrin, the products will elute just after the parent compound.
6. If degradation of either DDT or endrin exceeds 20 %, resalinize the injection port liner and/or break off ammeter from the front of the column. The degradation check solution is required each day in which analyses are performed.

$$\% \text{ Degrade of 4,4'DDT} = \frac{\text{Total DDT degradation peak area (DDE + DDD)}}{\text{Total DDT peak area (DDT + DDE + DDD)}} \text{ X } 100$$

$$\% \text{ Degrade of endrin} = \frac{\text{Total endrin aldehyde, EA + endrin}}{\text{Total endrin peak area + EA + E Karea}} \text{ X } 100$$

1. Results of the LFB are plotted over x-control chart to ensure that process variation is within statistical control.
2. If the real samples give positive results of DDT, DDE and DDD greater than PL and/or LOQ, the degradation check of DDT should be calculated.

3. The IPC solution and the GC degradation check solution may be prepared and analyzed as a single solution.

4. Analyze the instrument performance check sample and GC degradation check sample, and evaluate. If acceptance criteria are met, take remedial action. The following are suggested remedial actions which may improve method performance:

- Check and adjust GC operating conditions and temperature programming parameters.
- Clean or replace the splitless injector liner. Silanize a cleaned or new liner.
- Break off a short portion of the GC column from the end near the injector, or replace GC column. Breaking off a portion of the column will somewhat shorten the analyte retention times.
- Prepare fresh calibration solutions and repeat the initial calibrations.
- Replace any components in the GC that permit analytes to come in contact with hot metal surfaces.

References:

- Environmental Protection Laboratories, Canada, (Method 508), Organic Chlorine Pesticides in Environmental Samples, Preparation of Analytical Materials.
- Standard Methods for Examination of Water and Wastewater‖, 23st Edition, American Public Health Association (APHA), Washington 2017.
- U.S. Environmental Protection Agency. 1984. Method 608Organochlorine pesticides and PCBs. 40 CFR Part 136, 43321; Fed. Reg. 49, No. 209. 2. U.S. Environmental Protection Agency.
- Determination of pesticides and PCBs in industrial and municipal wastewaters; EPA 600/4-82-023, National Technical Information Serv., PB82.
- Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples; EPA-600/8-80-038. Health Effects Research Lab., Research Triangle Park, N.C.
- Alford-Stevens, A., et al. 1986. Characterization of commercial Aroclors by automated mass spectrometric determination of polychlorinated biphenyls by level of chlorination. Anal. Chem. 58:2014.
- Alford-Stevens, A. 1986. Analyzing PCBs. Environ. Sci. Technol. 20:1194.
- Quantitative PCB standards for election capture gas chromatography. J. Chromatog. Sci. 11:366.
- U.S Environmental Protection Agency. 1984. EPA Method Study - Method
- 608—Organochlorine Pesticides and PCBs; EPA-600/ 4-84-061.

(V.2) Organo-Chlorine Pesticides

(Gas Chromatography - Mass Spectrometer Detector)

Principle:

This method is applicable to the determination of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography in municipal and industrial discharges. A measured volume of sample is extracted serially with methylene chloride at a pH above 11 and again at a pH below 2. If necessary, the extract is then cleaned-up by passing through a glass column containing Florisil and elution with 5 % dichloromethane in hexane. The extract is dried, concentrated, and analyzed by GC/MS. Qualitative compound identification is based on retention time and relative abundance of three characteristic masses (m/z). Quantitative analysis uses internal-standard techniques with a single characteristic m/z. The method is applicable for determining the following pesticides that is shown in Table (V. 2).

Table (V. 2) Chlorinated pesticides that are analyzed by GC-MS

No.	Analyte	No.	Analyte
1	Alfa-BHC	10	4,4'-DDT
2	Beta-BHC	11	Endosulfan I
3	Gamma-BHC	12	Endosulfan II
4	Delta-BHC	13	Ensulfan sulfate
5	Aldrin	14	Methoxychlor
6	Dieldrin	15	Heptachlor
7	Endrin	16	Heptachlor epoxide
8	4,4'-DDE	17	Endrin ketone
9	4,4'-DDD	18	Endrin aldehyde

Equipment:

1. Sample containers with Teflon screw-cap and of capacity 60, 100, 250, 500 and 1000 ml.
2. Dispenser or equivalent of 10, 50 and 100 ml.
3. Gas chromatograph equipped with split-splitless-injector, mass detector and column: 30 m x 0.25 mm I.D.
4. Separatory funnels, 250 and 2000 ml.
5. Measuring flasks, 100 ml, class A.
6. Rotary evaporator.
7. Calibrated concentrator tube 10 ml.
8. Pasteur pipettes, 150 mm.
9. Calibrated syringe, 5, 10, 25, 100, 250, and 500 µl.
10. Calibrated syringe, 1, 2, and 5 ml.
11. Vials, 1, and 8 ml with caps.

12. Vials 14 ml with Teflon caps.
13. Muffle furnace range 1200 ºC.
14. Water bath range 120 ºC.
15. Nitrogen evaporation unit.
16. Technical balance of accuracy ± 0.01 g.
17. Analytical balance of accuracy ± 0.1 mg.
18. Drying cabinet range 200 ºC.
19. Glass wool, filter grade.
20. Clean up column, 300 mm x 10 mm.

Reagents:

N.B. The reagents, solvents and chemicals used are of the reagent HPLC grade chemicals.

1. Hexane, acetone dichloromethane, and propanol solvents.
2. Sulfuric acid.
3. Aluminum oxide, W200 neutral or basic (50-200 µm).
4. Silica gel (50-200 µm).
5. Florisil.
6. Sodium sulfate (anhydrous).
7. Hydrochloric acid.
8. Sodium hydroxide.
9. Pesticides mixture (2000 µg/ml) consisting of: Pentachloro nitrobenzene (PCNB), 98 % purity for use as internal standard and decachlorobiphenyl (DCB), 96 % purity for use as surrogate standard.

Procedure:

A. Standards preparations:

1. Stock standard solutions (2000 µg/ml). Stock standard solutions purchased as certified reference materials.
2. Internal standard solution. Prepare an internal standard of concentration 100 µg/ml of PCNB. Transfer the internal standard solution to a Teflon-sealed screw cap bottle and store at room temperature. Addition of 5 µl of the internal standard to 1.0 ml of sample extract results in a final internal standard concentration of 0.5 µg/ml.
3. Surrogate standard solution**.** Prepare a surrogate standard of concentration 500 µg/ml of DCB. Transfer the surrogate standard fortifying solution to a Teflon-sealed screw cap bottle and store at room temperature. Addition of 10 µl of the surrogate standard solution to a 1 liter sample prior to extraction results in a surrogate standard concentration in the sample of 5 µg/l and, assuming quantitative recovery of DCB, a surrogate standard concentration in the final extract of 5.0 µg/ml.

B. Extraction of water samples:

1. Mark the water meniscus level on the side of the sample bottle for later determination of sample volume.
2. Pour the entire sample into a two liters separatory funnel.
3. Rinse the sample with three 20 ml portions of dichloromethane; transfer each rinsing to the separatory funnel.
4. Acidify the water with 6 ml of 10 % hydrochloric acid.
5. Check pH to make sure that it is < 2, add more acid if required.
6. Shake the separatory funnel for 1 minute.
7. Allow the organic layer to separate for a minimum of 5 minutes.

(Note: If the emulsions interface between layers is more than one third the volume of solvent layer, add 10 ml of 6 N H_2SO_4 to the separatory funnel shake again and let stand for 2 minutes).

8. Assemble a drying funnel containing glass wool and 30 grams of sodium sulfate.
9. Rinse with three 10 ml portions of dichloromethane and discard these rinses.
10. Drain the extract through the sodium sulfate drying funnel into a 500 ml boiling flask.
11. Repeat the extraction procedure two more times with 60 ml of dichloromethane each time.
12. Add 10 ml of 10 N NaOH to the aqueous phase, check pH to make sure that it is > 10 and extract as described previously.
13. Drain dichloromethane extract through the same drying funnel, and collect in the same boiling flask.
14. Rinse the drying funnel with three 10 ml portions of dichloromethane; collect all the washings in the same 300 ml boiling flask.

(Note: If clean-up of the extract is necessary, as in case of soil and sediment extracts, the clean-up step is necessary. For water samples, a clean-up is hardly ever necessary. The professional judgment of the analyst is required to decide if clean-up is necessary).

15. If no cleanup is needed further, evaporate the extract to approximately 5 ml using a rotary evaporator, add 50 ml of hexane and concentrate the extract to approximately 2 ml. Concentrate the extract to 0.5 ml using a gentle stream of clean nitrogen with the tube placed in a warm water bath not exceeding 40 ^oC. Adjust the final volume to 1.0 ml with hexane, the extract is ready now for GC-MS analysis.

C. GC/MS analysis:

Establish the following GC/MS run conditions:

- Oven program: initial temperature 70 ^oC hold for 2 min and ramp temperature 20 ^oC/min to 240 ^oC.
- Mass detector temperature is 250 ^oC.
- Helium carrier gas of purity 99.999 % and at a flow rate of 1.2 ml /min are used.
- Injector temperature, 250 ^oC.
- Column: HP-5, (30 m x 0.32 mm I.D., df = 0.25 µm) or equivalent.

D. Calibration:

1. Using 3 of the calibration standards listed in the Table (V.3), perform a calibration to bracket the sample.

2. Inject one of the standards listed that gives a response closest to the sample responses, followed by the test sample extracts, followed by another injection of the selected standard to check the reproducibility of GC/MS response.

3. For each standard and sample analysis integrate the peaks, tabulate peak retention times and areas. The calibration standard is measured every set of samples (e.g. 10 or 20 samples).

4. If the calculated concentration of the standard differs more than 20 % of the true value, the reason must be found.

Table (V. 3) Calibration standards used for GC-MS

Concentrated solution, µg/ml	Aliquot, Ml	Dilution volume, ml	Calibration standard, µg/ml
200	1.0	10	20.0
200	0.5	10	10.0
200	0.25	10	5.0
20.0	1.0	10	2.0
20.0	0.5	10	1.0
5.0	1	10	0.5
10	0.25	25	0.1
1	0.25	25	0.01

- Calibration curve linearity is defined by the correlation coefficient (R^2). Acceptance criteria of r^2 value are the closer R^2 to 1 indicating a better quality of the predictions made through the calibration.
- If the calibration checks do not meet the laboratory criteria, re-analyze calibration standard, if not acceptable, prepare new calibration standards, if not acceptable, check the instrument response, if not acceptable, buy new references standard from different supplier, if not acceptable call for maintenance.
- All checks should be properly documented and adequately explained in the analytical report.

Calculation:

µg analyte/l sample = (A) (B) (D) (E)/(C) (F)

Where**:**

A = sample instrument response.

B = µg analyte/ml in the calibration standard.

C = calibration standard instrument response.

D = ml of the final extract (almost 1.0 ml).

E = dilution factor.

F = amount of samples analyzed in liters for aqueous samples.

Precautions:

1. All glassware that used in sample collection and pesticide residue analysis must be cleaned thoroughly. Clean glassware as soon as possible after use. Rinse with water or the solvent that was last used. Wash with soap, rinse with tap water, distilled water, redistilled acetone, and finally with pesticide-quality hexane.
2. As a precaution, glassware may be rinsed with the extracting solvent just before use. Heat heavily contaminated glassware in a muffle furnace at 400 ^{o}C for 15 to 30 min.
3. For reagents and chemicals, use reagent HPLC grade chemicals unless specified otherwise.

Quality control:

1. Use this method in accordance with the laboratory's QA program, following the applicable SOPs.
2. With each batch (e.g. 10 or 20 samples), analyze one reagent blank, one duplicate sample (if practical), and one spike or one reference material (if available). When there is no certified reference material available, analyze a control standard (a standard prepared from different suppliers, lot numbers or stocks). Each time a set of samples is extracted or reagents are changed, a blank must be analyzed. No detectable levels of analytes should be found in blanks. The % RSD of duplicate results should not exceed ± 20 %.
3. Control standard or reference material results should fall within designated control limits or 80 and 120 % recovery whichever is greater.
4. Spike recoveries should fall within 80 and 120 % or the limits established by the method performance data whichever is greater.
5. Check the accuracy of the 0.1 to 2 µg/l range by using a 0.5 µg/l standard solution.
6. The mass detector performance is checked by measuring the ion abundance criteria for injection 2 µl of 50 ng/µl DFTPP and the criteria are shown as the following in Table (V. 4):

Table (V. 4) Ion abundance criteria for injection of DFTPP (2 µl of 50 ng/µl)

Mass	Ion abundance criteria
51	30-60 percent of mass 198
68	Less than 2 percent of mass 69.
70	Less than 2 percent of mass 69.
127	40-60 percent of mass 198.
197	Less than 1 percent of mass 198.
198	Base peak, 100 percent relative abundance.
199	5-9 percent of mass 198.

275	10-30 percent of mass 198.
365	Greater than 1 percent of mass 198.
441	Present but less than mass 443.
442	Greater than 40 percent of mass 198.
443	17-23 percent of mass 442.

References:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017.Method 6632.
- Environmental Protection Laboratories, Canada, (Method 611.1), Rev. 0 Organochlorine Pesticides in Environmental Samples, Preparation of Analytical Materials.

(V.3) Poly Chlorinated Biphenyls (PCBs)

(Gas Chromatography - Electron Capture Detector, ECD)

Principle:

A measured volume of the test water sample, usually 1 liter, is serially extracted with methylene chloride using a separatory funnel. The extract is dried, concentrated (if necessary), and exchanged into a solvent compatible with the cleanup or the chromatographic method used for determination. The method is applicable to the isolation and concentration of water-insoluble and slightly water-soluble persistent organics.

Equipment:

1. Separatory funnel, 2-liter, with polytetrafluoroethylene (PTFE) stopcock.
2. Drying column, 20 mm ID Pyrex® chromatographic column with Pyrex® glass wool at bottom and a PTFE stopcock.
3. Kuderna-Danish (K-D) apparatus or rotary evaporator.
4. Concentrating tube, 10-ml, graduated "A ground-glass stopper is used to prevent evaporation of extracts".
5. Evaporation flask, 500-ml attach to concentrator tube with springs, clamps, or equivalent.
6. Chromatographic column, 300 mm x 10 mm ID, with polytetrafluoroethylene (PTFE) stopcock.
7. Beakers, appropriate sizes.
8. Reagent bottle, appropriate sizes.
9. Muffle furnace, capable of maintaining 400 ^{o}C.
10. Vials, glass, 10-ml and 25-ml capacity, with PTFE-lined screw caps or crimp tops.
11. Snyder column, three-ball macro.
12. Springs, 1/2 inch.

Reagents:

1. Reagent grade chemicals shall be used in all tests. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.
2. Organic-free reagent water.
3. Sodium hydroxide solution (10 N), Dissolve 40 g of NaOH in organic-free reagent water and dilute to 100 ml.
4. Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating to 400 ^{o}C for 4 hours in a shallow tray.

5. Sulfuric acid solution (1:1 v/v), H_2SO_4. Slowly add 50 ml of H_2SO_4 (sp. gr. 1.84) to 50 ml of organic-free reagent water.
6. Extraction/exchange solvents. All solvents must be pesticide quality or equivalent, organic-free reagent water:
   - Methylene chloride, CH_2Cl_2, boiling point 39 ^oC.
   - Hexane, C_6H_{14}, boiling point 68.7 ^oC.
   - Propanol, $CH_3CH(OH)\ CH_3$, boiling point 82.3 ^oC.
   - Cyclohexane, C_6H_{12}, boiling point 80.7 ^oC.
   - Acetonitrile, CH_3CN, boiling point 81.6 ^oC.
7. Granular Florisil, for column cleanup procedure.
8. Deactivation/Activation of Florisil. Deactivation of Florisil - for cleanup of phthalate esters. To prepare for use, place 100 ± 10 g of Florisil into a 500-ml beaker. Heat to 140 ^oC for approximately 16 h. Transfer to a 500-ml reagent bottle. Tightly seal and cool to room temperature. Add 3 ± 0.1 ml of organic-free reagent water. Mix thoroughly by shaking or rolling for 10 min and let stand for at least 2 h. Keep the bottle sealed tightly
9. Activation of Florisil for all cleanups other than phthalate esters. It is advisable to treat both Florisil A and Florisil PR prior to use to drive off any moisture adsorbed during storage and handling. Heat the Florisil in a glass container loosely covered with aluminum foil in an oven at 130 ^oC overnight. Cool the Florisil in a desiccator before use.
10. Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400 ^{o} C for 4 hours in a shallow tray.
11. Eluting solvents. All solvents must be pesticide quality or equivalent.
    - Diethyl ether, $C_2H_5OC_2H_5$.
    - Pentane, $CH_3(CH_2)_3CH_3$.
    - Hexane, C_6H_{14}.
    - Methylene chloride, CH_2Cl_2.
    - Acetone, CH_3COCH_3.
    - Petroleum ether (boiling range 30-60 ^oC).
    - Toluene, $C_6H_5CH_3$.
    - Propanol, $(CH_3)_2\ CHOH$.

Procedure:

1. Using a 1-liter graduated cylinder, measure 1 liter (nominal) of the test sample.
2. Check the pH of the sample with wide-range pH paper and adjust the pH, if necessary, to the pH 5 - 9, using 1:1 (v/v) sulfuric acid or 10 N sodium hydroxide.
3. Quantitatively transfer the sample from the graduated cylinder (or sample bottle) to the separatory funnel. Use 60 ml of methylene chloride to rinse the cylinder (or bottle) and transfer this rinse solvent to the separatory funnel.

4. Seal and shake the separatory funnel vigorously for 1 - 2 minutes with periodic venting to release excess pressure.
5. Allow the organic layer to separate from the water phase for a minimum of 10 minutes, collect the solvent extract in an Erlenmeyer flask.
6. Repeat the extraction two more times using fresh portions of solvent, collect, combine and label the combined extract.
7. Perform the concentration (if necessary) using the Kuderna-Danish Technique or rotary evaporator.
8. If a solvent exchange by hexane is required, momentarily remove the Snyder column, add 50 ml of the exchange solvent, a new boiling chip, and reattach the Snyder column. Alternatively, pour the exchange solvent into the top of the Snyder column while the concentrator remains on the water bath.
9. Remove the Snyder column; rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml exchange solvent, adjusted to 10.0 ml with the last solvent used.

Table (V. 5) The analyte groups

Phthalate esters	Organochlorine pesticides
Chlorinated hydrocarbons	Organophosphates
Nitrosamines	Organophosphorus pesticides
Nitro aromatics	Aniline and aniline derivatives
Haloethers	PCBs

10. The extract may now be analyzed for the target analytes using the appropriate determination technique.
11. If analysis of the extract will not be performed immediately, stopper the concentrator tube and store in a refrigerator. If the extract will be stored longer than 2 days, it should be transferred to a vial with a Teflon-lined screw-cap or crimp top, and labeled appropriately.

Method quality control:

Any reagent blanks, matrix spikes, or replicate samples should be subjected to exactly the same analytical procedures as those used on actual sample.

1. Florisil, a registered trade name of U. S. Silica Co., is a magnesium silicate with basic properties. It is used to separate analytes from interfering compounds prior to sample analysis by a chromatographic method.
2. Florisil cleanup may be accomplished using a glass chromatographic column packed with Florisil or using solid-phase extraction cartridges containing Florisil.
3. This method includes procedures for cleanup of sample extracts containing the following analyte groups as Shawn in Table (V. 5).

Procedure:

A. Preparation of cartridge:

1. Arrange the cartridges on the manifold in the closed-valve position.

2. Turn on the vacuum pump and set the vacuum to 10 in (254 mm) of Hg.
3. Condition the cartridges by adding 4 ml of hexane to each cartridge.
4. Slowly open cartridge set-up and conditioning. The cartridge valves are opened to allow hexane to pass through the sorbent beds to the lower frits.
5. Allow a few drops per cartridge to pass through the manifold to remove all air bubbles. Close the valves and allow the solvent to soak the entire sorbent bed for 5 minutes. Do not turn off the vacuum.
6. Slowly open the cartridge valves to allow the hexane to pass through the cartridges. Close the cartridge valves when there is still at least 1 mm of solvent above the sorbent bed.
7. Do not allow cartridges to become dry. If cartridges become dry, repeat the conditioning step.

B. Handling of sample extracts:

Most sample extracts are concentrated to a smaller volume prior to the use of Florisil cleanup. Reduce the sample extract volume to 10 ml prior to cleanup for organo chlorine pesticides, organophosphorus pesticides, haloethers, organo-phosphates, and PCBs.

C. Cartridge for organochlorine pesticides and PCBs:

1. Using 1 g Florisil cartridges, condition the cartridges with hexane, as described.
2. Transfer 1.0 ml of the extract to the cartridge. Open the cartridge valve to allow the extract to pass through the cartridge bed at approximately 2 ml/minute.
3. When the entire extract has passed through the cartridge, but before the cartridge becomes dry, rinse the sample vial with an additional 0.5 ml of hexane, and add the rinse to the cartridge to complete the quantitative transfer.
4. If there is no need to separate the organochlorine pesticides from the PCBs, add 9 ml of acetone/hexane (10/90, v/v) to the cartridge.
5. Turn on the pump and adjust the pressure to 10 inches (254 mm) of Hg.
6. Allow the solvent to soak the sorbent bed for 1 minute or less. Slowly open the cartridge valve and collect the eluate into the collection vial.
7. As needed, perform a solvent exchange and adjust the final volume of the eluent to the volume listed in the assay method.

References:

- Environmental Protection Laboratories, Canada, (Method: 3510C, 3620B, 8082) Poly Chlorinated Biphenyls Samples, Preparation of Analytical Materials.
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition,
- American Public Health Association (APHA), Washington 2017 (Method 5520 (C).

(VI) Polycyclic Aromatic Hydrocarbons (PAH's)

(VI.1) Polycyclic Aromatic Hydrocarbons (PAH's)

(High Pressure Liquid Chromatography - Fluorescence Detector)

Principle:

A measured volume of the sample (approximately 1 liter), is extracted with methylene chloride using a solid phase extraction. The organic extract is dried and exchanged to acetonitrile during concentration to a volume of 5 ml. The extract is subjected to HPLC measurements using a fluorescence detector. The method is applicable to the determination of the following PAH's (Table VI.1) in finished drinking water, raw water and drinking water during intermediate stages of treatment.

Table (VI.1) Poly cyclic aromatic hydrocarbon compounds

No.	Compound	No.	Compound
1.	Acenaphthylene	2.	Anthracene
3.	Benzo(a)anthracene	4.	Benzo(a)pyrene
5.	Benzo(b)fluoranthene	6.	Benzo(g,h,i)perylene
7.	Benzo(k)fluoranthene	8.	Chrysene
9.	Dibenzo(a,h)anthracene	10.	Fluoranthene
11.	Fluorene	12.	Indeno(1,2,3-cd)pyrene
13.	Naphthalene	14.	Phenanthrene
15.	Pyrene		

Definitions:

1. Laboratory duplicates (D1, D2). Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of duplicates give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
2. Laboratory reagent blank (B). An aliquot of reagent water that is treated exactly as the sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The blank is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
3. Laboratory fortified blank (LFB). An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

Equipment:

1. Micro syringes of different volumes; 10 , 25 , 50, 100, 250 and 1000 µl.
2. Measuring flasks of different volumes; 1, 5, 10, 25, 50, 100, 500, 1000 and 2000 ml.
3. Amber glass bottles with Teflon septa screw cap 40 and 20 ml.
4. Disposable glass transfer pipettes (Pasteur), 1 ml.

5. Flask 250 and 500 ml.
6. Auto sampler vials, with Teflon crimp caps, or screw cap.
7. Balance, analytical, capable of accurately weighing to the nearest 0.0001 g.
8. Standard solution storage containers - 4 ml vial round, amber glass bottles with Teflon-lined caps.
9. HPLC system, capable for inject of 1000 µl and equipped with a fluorescence detector, an auto- sampler, a column C-18 (4.5 x 250 mm, bonded fused silica column, 4 µm), data system for acquisition and data analysis for measuring peak areas and retention times.
10. Fluorescence detector is used for excitation at 280 nm and emission greater than 389 nm cutoffs (Schoeffel FS970 or equivalent). Fluorometer should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector (the manufacturer application notes should be followed).
11. Rota-vapor with vacuum.
12. Oven up to 450 °C.
13. Refrigerator.
14. Calibrated glass thermometer.

Reagents:

1. Acetonitrile, free of PAH's.
2. Acetone, free of PAH's.
3. Methanol, free of PAH's.
4. Reagent water.
5. Sodium thiosulfate, high purity grade.
6. PAH's calibration mixture contains the listed analytes.
7. Methylene chloride, free of PAH's.
8. Sodium sulfate anhydrous stored in a brown glass bottle (heat treatment in a shallow tray at 400 °C for a minimum of four hours to remove interfering organic substances.

Procedure:

***A.* Glassware washing:**

1. Wash all glassware used in PAH's analysis by rinsing with hot tap water, then with a carbon and phosphorus free detergent, then with hot tap water and finally with reagent water.
2. Allow the bottles and seals to air dry, then place in a 400°C oven (except the standard volumetric glassware) for 1 hour, or wash with acetone and then allow cooling at ambient temperature.
3. Before starting extraction, wash the glasses with methylene chloride and wash the measuring flasks with acetone then with acetonitrile.

***B. Sample stabilization*:**

If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample in the sample bottle prior to sample collection.

C. Sample collection:

1. Collect the samples with at least one sample in duplicate per batch (batch = 20 samples). Don't allow sample over flow.
2. Analytes present in aqueous refrigerated or freezed samples are stable for 7 days and the refrigerated organic extract is stable for 30 days.

D. Extraction and measurements:

1. Add 60 ml methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to a separatory funnel and extract the sample by vigorous shaking the funnel for two minutes with periodic venting to release excess pressure.
2. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 500 ml conical flask.
3. Add a second 60 ml volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in an Erlenmeyer flask.
4. Perform a third extraction in the same manner.
5. Add about 5 g anhydrous sodium sulfate to dry the extract.
6. Stir the flask to dry extract and allow sitting for 15 minutes.
7. Decant the methylene chloride extract into the Rota vapor flask.
8. Rinse the remaining sodium sulfate with two 25 ml portions of methylene chloride and decant the rinses into the Rota vapor flask.
9. Set the Rota vapor water bath temperature at 42-44°C till the volume of extract reach to about 5 ml. Add 3 ml acetonitrile.
10. Concentrate the volume to about 1 ml using Rota vapor at 42-44 °C under vacuum, adjust the volume to 5.0 ml with acetonitrile and inject 20 µl of the sample extract.
11. Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest.
12. . Follow the manufacturer application notes for instrument conditions**.**

Precautions:

1. Ensure that all samples are preserved in a refrigerator at 4°C.
2. Impurities contained in the mobile phase usually account for the majority of the analytical problems.

Interferences:

Solvent blanks are analyzed with each analytical batch of samples. An interference-free solvent is a solvent containing no peaks yielding data at greater than or equal to the LOQ and at the retention times of the analytes of interest.

References:

- Environmental Protection Laboratories, Canada, Method 550 in Environmental Samples, Preparation of Analytical Materials.
- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017 (Method 5520 (C).

(VI.2) Polycyclic Aromatic Hydrocarbons (PAHs)

(Gas Chromatography - Flame Ionization Detector, FID)

Principle:

A measured volume of water test sample is extracted with methylene chloride. The extract is dried, concentrated, and measured using gas chromatography with flame ionization detector. Method cleanup is used for the elimination of the interferences.

Equipment:

1. Separatory funnel, 2-liter with Teflon stopcock.
2. Drying column, chromatographic.
3. Concentrator tube, Kuderna-Danish, 10 ml.
4. Evaporative flask, Kuderna-Danish, 500 ml.
5. Vials, 10 to 15 ml amber glass, with Teflon-lined screw cap.
6. Water bath, use bath in a hood.
7. Analytical balance, capable of accurately weighing 0.0001 g.
8. Gas chromatograph: An analytical system complete with temperature programmable suitable for on-column or splitless injection when capillary columns are used.
9. All required accessories including syringe, analytical columns, and gases.
10. Detector, flame ionization (FID).

Reagents:

1. Reagent water obtained from a water purification system.
2. Sodium thiosulfate granular.
3. Cyclohexane, methanol, acetone, methylene chloride, pentane, pesticide quality or equivalent.
4. Sodium sulfate anhydrous.
5. Silica gel, 100/200 mesh, desiccant activated for at least 16 h at 130 ^{o}C in a shallow glass, loosely covered with foil.
6. Stock standard solutions. Prepared from pure standard PAH's by accurately weighing about 0.0100 g, dissolving in a pesticide quality acetone or other suitable solvent, and diluting to volume in a 10-ml volumetric flask (1.0 µl ≡ 1.00 µg compound).
7. Calibration standards. Prepared at a minimum of three concentration levels for each compound by adding appropriate volume of one or more stock standards to a volumetric flask. To each calibration standard or standard mixture, add a known constant amount of one or more internal standards, and dilute to volume with acetone. Prepare one calibration standard at a concentration near, but above, the MDL and others corresponding to the expected range of sample concentration or defining the working range of the GC-FID system.

Procedure:

N.B. Pour entire sample into a 2-liter separatory funnel and extract as directed without any pH adjustment.

A. Extraction:

1. Separatory funnel for extraction, normally 2 liter.

2. Pour entire sample into a 2-liter separatory funnel.

3. Pipette 1.0 ml surrogate standard solution into separatory funnel and mix well.

4. Add 60 ml methylene chloride to the sample bottle, seal, and shake for 30 s to rinse inner surface. Transfer the solvent to a separatory funnel and extract sample by shaking for 2 min with periodic venting to release excess pressure.

5. Let organic layer separate from water phase for a minimum 10 min. If emulsion interface between layers is more than one–third the volume of the solvent layer, use mechanical techniques to complete phase separation by stirring, filtering emulsion through glass wool, centrifuge, or other physical methods.

6. Collect methylene chloride in a 250 ml Erlenmeyer flask.

7. Add a second 60 ml of methylene chloride to sample bottle and repeat extraction procedure, combining extracts in the Erlenmeyer flask.

8. Perform the third extract at the same manner. Pour combined extract through solvent–rinsed drying column containing at least 10 cm anhydrous sodium sulfate or more, and collect extract in concentrator.

9. Rinse Erlenmeyer flask and column with 20-30 ml methylene chloride to ensure quantitative transfer.

10. Add 1-2 clean boiling chips to the evaporative flask and attach a-three-ball Snyder column. Pre-wet Snyder column by adding about 1 ml methylene chloride to the top.

11. Place K-D apparatus on a hot water bath (60-65 ºC) in a hood so that concentrator tube is partially immersed in the hot water and that concentrator tube is partially immersed in the hot water and the entire lower rounded surface is passed with hot vapor.

12. Adjust vertical position of apparatus and water temperature as required to complete concentration in 15-20 ml. At proper rate of distillation the column balls.

13. With the apparent volume of liquid reaches 1 ml, remove K-D apparatus and let drain and cool.

14. Remove Snyder column, and rinse flask and its lower joint into the concentrator tube with 1-2 ml methylene chloride, preferably using a 5 ml syringe for this operation.

15. Stopper concentrator tube and store refrigerated, if further processing will not be done immediately.

16. If extract is to be stored long than 2 d, transfer to a Teflon-sealed screw cap vial and protect from light.

B. Cleanup and separation:

N.B. Before using silica-gel cleanup technique, exchange extracts solvent to cyclohexane.

1. Add 1 to 10 ml sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube.
2. Add 4 ml cyclohexane and attach a two-ball micro-Snyder column.
3. Pre-wet column by adding 0.5 ml methylene chloride to the top.
4. Place micro-K-D apparatus on a boiling (100 ^{o}C) water bath so that concentrator tube is partially immersed in hot water.
5. Adjust vertical position of apparatus and water temperature so as to complete concentration in 5 to 10 min.
6. At proper rate of distillation, the column balls actively chatter but are not flooded.
7. When apparent volume of liquid reaches 0.5 ml, remove K-D apparatus and let drain and cool for at least 10 min.
8. Adjust extract volume with a minimum amount of cyclohexane to about 2 ml.

C. Cleanup of silica-gel column:

1. Make slurry of 10 g activated silica gel in methylene chloride and place in a 10 mm ID chromatographic column.
2. Tap column to settle silica gel and elute with methylene chloride.
3. Add 1 to 2 cm anhydrous sodium sulfate to top of silica gel.
4. Pre-elute with 40 ml pentane. Elute at rate of about 2 ml/min.
5. Discard elute and just before exposure of sodium sulfate layer to the air, transfer all cyclohexane sample extract onto column using a an additional 2 ml cyclohexane, then add 25 ml pentane and continue elution.
6. Next elute column with 25 ml methylene chloride/pentane (4+6) into a 500 ml K-D flask equipped with a 10 ml concentrator tube.

D. Gas chromatographic measurements:

1. Inject 3 to 4 µl of the extract solution into the fused silica capillary column (split or splitless).
2. Always inject the same volume.

E. Calibration:

Calibrate system daily, using either external or internal standard procedure.

Calculation:

Concentration (µg/l) = (As) (Is) / (Ais) (RF) (Ve)

As = area of characteristic m/z for compound or surrogate standard to be measured.

Ais = area of characteristic m/z for internal standard.

Is = amount if internal standard added to each extract, µg.

Ve = volume of water extracted, liter.

RF = response factor.

Interferences:

1. Some interference in GC analyses occurs due to sample, solvent, or carrier gas contamination, or because of the injection of large amounts of a compound and nonlinear response of the detector.
2. Interferences due to the sample can be removed by auxiliary cleanup techniques.
3. A magnesia-silica gel column cleanup and separation procedure is used for this purpose. Such cleanup usually is not required for potable waters.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method (6440 B).

(VII) Microbiological Examination

(VII.1) Membrane Filter (MF) Technique

(General Techniques)

Introduction:

This method includes procedures for determining the content of some types of bacteria groups in water. It can be used to test relatively large sample volumes, and usually yields numerical results more rapidly than multiple-tube fermentation procedure. MF procedure involves filtering a sample aliquot through a 0.45 μm membrane filter. The filter is placed grid-side up onto a petri dish containing a selective media and the plate incubated under specific conditions for a specified temperature and time. The media used to culture the bacteria and the incubation conditions, temperature and time are specific to the type or group of bacteria to be tested. Following incubation, the number of bacterial colonies on the plate is counted and this number used to calculate the bacterial density.

Bacterial groups:

1. *Total coliform (TC):*

Comprise all aerobic and facultative anaerobic gram-negative, non-spore forming, rod-shaped bacteria that produce a colony with a golden green metallic sheen within 22-24 hours at 35±0.5 °C on an Endo type medium containing lactose. Selective media used; M-Endo agar LES, selective temperature; 35±0.5 °C/22-24 h, (Standard Methods, Method no. 9222B).

2. *Fecal coliform (FC):*

Comprise all thermo tolerant gram-negative non-spore forming bacilli that ferment lactose with gas production at 44.5±0.2°C within 24 hrs. Selective media used; M-FC agar, selective temperature 44.5 °C/24 h, (Standard Methods, Method no. 9222D).

A. Escherichia coli (E. Coli):

It is a member of the indigenous fecal flora of warm-blooded animals. The presence of E. coli in water is considered a specific indicator of fecal contamination with possibility of the presence of enteric pathogens. Selective media used ; M-TEC agar, selective temperature 35±0.5 °C/48 h, (Standard Methods, Standard Methods, Method no. 9222 G).

B. Fecal streptococci (F.S.):

Gram-positive cocci occurring in pairs are indicators of fecal pollution; the normal habitat is the intestines of man and animal. Selective media used; M-Enterococcus agar, selective temperature 35 °C/48 h, (Standard Methods, Method no. 9230 C).

C. Pseudomonas aeruginosea (P.A.):

Gram-negative non-lactose fermenting, rod shaped bacteria. It found in the of whirlpools which usually are not cleaned drained and refilled after each use.

Selective media used; R2A agar is incubated at 37±0.5 °C for 24±0.5 h. Aseptically

transfer the membrane to M-PA agar and incubate at 35±0.5 °C for another 24 h. Random and atypical colonies can be verified use confirmatory test by using milk agar and incubate at 35±0.1 °C for 24 h till the appearance a yellowish to green pigment.

Interferences:

High levels of turbidity may interfere with colony counts and may not permit testing of a sample volume large enough to yield significant results. Low coliforms estimates may be caused by the presence of high numbers of non-coliform bacteria or of toxic substances.

Sample collection and handling:

1. Collect 500 ml to 1 liter of the test sample in a sterile plastic or non-reactive borosilicate glass bottles (containing 0.4 ml of sterile 10 % sodium thiosulfate for drinking water). The container should not be fully filled to allow aerobic bacteria to grow. Water samples should be analyzed soon after collection to avoid unpredictable changes. If samples can't be analyzed within 1 hour after collection, use an insulated iced cooler for transportation to the lab. Never freeze samples.
2. Transport and store samples at 1 - 8 °C. Do not leave at room temperature for more than 1 hour. The time elapsing between collection and examination must never be longer than 30 hours.
3. The time elapsing between collection and examination must never be longer than 30 hours.
4. All labels should carry complete and accurate identification information.

Reagents:

1. Buffered water with KH_2PO_4 and $MgCl_2$. Add 1.25 ml of stock phosphate buffer solution and 5 ml of magnesium chloride solution to 1 liter of reagent water.

2. Ethyl alcohol, 95 %.

3. Magnesium chloride solution. Dissolve 38 g magnesium chloride in reagent water and dilute to one litre with reagent water. Shelf life: two years.

4. Phosphate buffer, stock solution. Dissolve 34 g potassium dihydrogen phosphate (KH_2PO_4) in 500 ml reagent water; adjust to pH 7.2 ± 0.5 with 1 N sodium hydroxide (NaOH) and dilute to 1 liter with reagent water. Shelf life: two years.

5. Rosolic acid, 1 % . Add 0.1 g rosolic acid to 10 ml of 0.2 N NaOH solution. Shelf life: one week.

6. Sodium hydroxide (NaOH), 1 N. Dissolve 40 g of sodium hydroxide solid in reagent water. Dilute to one litre with reagent water. Shelf life: indefinite**.**

7. Sodium hydroxide (NaOH), 0.2 N . Dissolve 8 g of solid sodium hydroxide in reagent water. Dilute to one litre with reagent water. Alternately dilute 200 ml of 1 N solution to one litre. Shelf life: indefinite.

8. Sodium thiosulfate, (10%). Dissolve 100 g of solid sodium thiosulfate in reagent water and dilute to one litre with reagent water. Shelf life: two years.

9. Bacto triphenyltetrazolium chloride (TTC), 1 %. Add 1 g of TTC to 100 ml of reagent water, dissolve, filter and sterilize through a 0.2 μm filter. Pour into a sterile dilution bottle and store in fridge at 2-10 °C. Shelf life: one year.

Note: Use reagent grade chemicals unless specified otherwise.

Media preparation:

1. *m-Endo Agar (for TC):*

Mix 51 g of the media with 1 liter of reagent water. Add 20 ml of ethanol/litre and heat to boiling. Cool to 44-50 °C and dispense into pre-sterilized petri dishes. Store plates in dark at 2-10 °C.

2. *m-FC Agar (for FC):*

Mix 52 g of the media with 1 liter of reagent water. When the media almost comes to a boil, add 10 ml of 1 % rosolic acid solution per litre. Continue to boil for 1 min, cool and dispense into pre-sterilized petri dishes. Store in the dark at 2 - 10 °C.

3. *m-TEC agar (for E. coli):*

Mix 46.5 g of the media in 1 liter of reagent grade water, sterilize by boiling for 1 min and then pour in plates.

4. *m-Enterococcus agar (for FS):*

Suspend 42 g of the media in 1 liter of reagent grade water, sterilize by boiling for 1 min and then pour in plates.

5. *m-PAC agar (for P. A.):*

Suspend 35 g of the media in 1 liter reagent grade water, sterilize by boiling for 1 min and then pour in plates.

Equipment:

1. Filter holder, stainless steel manifolds may be used**.**
2. Membrane filters, cellulose acetate/nitrate membranes: 0.45 μm size.
3. Filter flasks.
4. Vacuum source.
5. Sample bottles, autoclavable polypropylene 500 and 1000 ml bottles.
6. Dilution bottles, 30 and 90 ml blanks of sterilized buffered water**.**
7. Rinse bottles, 1 liter glass bottles of sterilized buffered water or use 500 ml pre-sterilized plastic jet.
8. Pipettes, 1 ml and 10 ml sterilized pipettes or fixed micropipettes adjusted at different volumes.
9. Graduated cylinders, 100 ml.
10. Forceps, round tipped without corrugations on the inner sides.
11. Small beaker**.**

12. Bunsen burner, to flame alcohol dipped forceps.
13. Incubators, maintained at 35 °C, 41.5 °C and 44.5±0.5 °C.
14. Water bath (or large incubator), maintained at 44.5±0.2 °C.
15. Colony counter, Quebec Colony Counter.
16. Autoclave, sterilized 15 minutes at 121°C.
17. Petri-dishes, 100 mm diameter (SPC), 60x15 or 50x12 mm.
18. pH-Meter, to determine pH of media and buffered water.
19. Glassware bottles.

Washing and sterilization:

1. All glassware is washed according to the standard methods.
2. Dilution blanks and rinse bottles are filled with appropriate amount of buffered water and are sterilized in an autoclave at 121°C for 15 min. Caps should be tightened soon after removal from the autoclave.
3. Filter flasks are double paper bagged and sterilized in an autoclave at 121°C for 15 min.
4. Pipettes in metal containers are sterilized in ovens with a temperature of approximately 170 °C for no less than 2 hours.
5. Sample bottle preparation.
6. Use 500 ml to 1 liter wide mouthed heat resistant plastic bottles with screw on caps. Wash sample bottles thoroughly with a detergent and rinse well with distilled water.
7. A 10 % sodium thiosulfate solution is used as a dechlorinating agent. For 1litre bottles, add 0.8 ml of 10 % solution. For 500 ml bottles, add 0.4 ml of 10 % solution.
8. Recap loosely and autoclave for 15 minutes at 121 °C. Tighten caps when the bottles have cooled down.

Procedure:

A. Sample volume selection:

1. Record the temperature of samples upon receipt; do not contaminate by Placing a thermometer inside.
2. All samples and dilutions must be mixed thoroughly by vigorously shaking up and down (about 30 complete times).
3. Select the proper dilutions, so that the total number of colonies on a plate will be between 20 and 80 colonies and not more than 200 colonies of all types per membrane. Use direct volumes as shown in Table (VII.1).
4. Mark each plate on the top with sample number and dilution.
5. Only remove pipettes from sterile container, just prior to using and use blowout pipettes for all dilutions.

Table (VII.1) Suggested sample volumes for membrane filter test

Water source	Volume (X) to be filtered, ml								
	1000	100	50	10	1	0.1	0.01	0.001	0.0001
Drinking water	X	X							
Swimming pools		X							
Wells, springs		X	X	X					
Lakes, reservoirs		X	X	X					
Water supply intake				X	X	X			
Bathing beaches				X	X	X			
River water					X	X	X	X	
Chlorinated sewage					X	X	X		
Raw sewage						X	X	X	X

Note: Control plates are required using sterile buffered water as test solution. After running through dilution series and plating all filters, pour sterile buffered water through funnels and filters and place filters on plates for each agar type used.

B. Sample filtration:

1. Set up 6 filter funnels on a manifold hooked up to a vacuum source.
2. Using sterile forceps flamed in ethanol; place a sterile filter over porous plate of receptacle, grid side up.
3. Carefully place funnel unit over receptacle and lock it in place.
4. Filter sample under vacuum. Pour about 30 ml dilution blanks with sample added through filter.
5. With filter still in place, rinse funnel in a circular motion 4 times with a total of 20 - 30 ml of sterile buffered water from a sterile rinse bottle.
6. Unlock remove funnel and immediately remove filter with sterile forceps. While holding the filter, replace the funnel and turn off vacuum. Place filter on agar with a rolling motion to avoid air entrapment.
7. Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water.
8. Incubate the control membrane culture under the same conditions as the sample.
9. Incubate TC plates at 35±0.5 °C for 24 h, FC plates at 44.5±0.2 °C for 24 h, E. coli plates at 35±0.5 °C for 48 h, FS plates at 35±0.5 °C for 48 h, and PA plates at 35±0.5 °C for 24 h. and milk agar platesat 35±0.1 °C for 24 h
10. Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination.

Calculation and reporting:

1. Report counts per 100 ml.
2. Cultural characteristics.
3. TC red colonies with metallic sheen.

4. FC blue colonies.
5. E. coli blue colonies.
6. FS red colonies.
7. PA greenish brown colonies.
8. Compute counts from filters with 20-80 colonies and not more than 200:

Count/100 ml = colonies counted x 100/ml sample filtered

9. Use an aid such as the Quebec Colony Counter.
10. Count all colonies on selected plates.

Quality control:

1. Check sterility of each media, membrane filters, buffered water in rinse bottle, glassware and equipment once during each series of samples using sterile buffered water as the sample.
2. Pour sterile buffered water through funnels and filters and place filters on each of the agar types being used for the samples.
3. Perform duplicate analysis on at least one sample per test run. Duplicate results must be within the same order of magnitude (e.g., 10 - 99).
4. To ensure quality control conformance, there must be no colony growth for the method blanks or the test is invalid.
5. Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water.

(VII.2) Total Bacterial Count
(*Standard Plate Count)*

Introduction:

Standard plate count provides an approximate enumeration of total numbers of viable bacteria that may yield useful information about water quality and may help with interpretation of coliform results.The standard plate count test involves adding 0.1 ml of a sample or a sample dilution to a petri dish. Standard plate count media is then added to the petri dish and the plate (petri dish, media and sample aliquot) is incubated for 48±3 h at 35±0.5°C. Following incubation, the numbers of bacterial colonies on the plate are counted and this number used to calculate the bacterial density.

Media:

Standard plate count agar (tryptophane glucose yeast agar) is used for pour and spread plate methods.

Media preparation:

Standard plate count agar:

Mix 23.5 g of the media with 1 liter reagent water in a flask. Boil and autoclave for 15 min. at 121°C. Maintain sterile media in a water bath between 44-46 °C. Make fresh daily.

Procedure:

Dilution procedure

1. Prior to setup for dilutions of sample and membrane filter technique, checks should be done on incubator and water bath temperatures. Prior to testing for fecal coliforms the water bath should be filled with RO water and turned on. The temperature must be monitored to ensure stability at 44.5±0.2°C. Adjust dial in front of bath accordingly so that temperature is maintained within the range. Prior to testing, the incubator temperature must be monitored to ensure maintenance of a temperature of 35.0±0.5°C.
2. Prepare serial dilutions of each sample. Select dilutions so that the total number of colonies on a plate will be between 30 and 300 for SPC. For example: If the SPC is suspected to be as high as 3000, prepare plates of a (1:10) dilution.
3. All samples and dilutions must be mixed thoroughly by vigorously shaking up and down (about 30 complete times).
4. Pour control plates using 1 ml of sterile buffered water.
5. Mark each plate on the bottom side with sample number and dilution.
6. Prepare serial dilutions as 1/10, 1/100, 1/1000, 1/10000, 1/100000, 1/1000000, and 1/10000000.
7. Use sterile blowout pipettes for initial and subsequent transfers.

8. Begin with 90 ml blanks and use a separate sterile pipette for transfers from each serial dilution.
9. Allow the pipette tip to drop 2-4 cm into sample and touch tip to the sample bottle lip on the way out.
10. Use this same technique for each 90 ml serial dilution blank.
11. Recap and shake each blank 30 times before pipetting the next dilution out
12. Pour plate of standard plate count.
13. A level benchtop that has been disinfected in a draft free area is required.
14. Ensure that area is always washed down prior to and after analysis and well lighted.
15. Mark each plate on the bottom side with sample number and dilution.
16. Prepare the serial dilutions of sample as shown in diagram.
17. Use sterile pipettes for initial and subsequent transfers (blow out type).
18. Use a separate sterile pipette for transfers from each serial dilution.
19. Blow remaining sample from pipette and touch tip of pipette once against a dry spot on the petri dish bottom.
20. Pipette 1.0 ml of the appropriate dilution into an empty sterile petri dish, 100x15 mm.
21. Pour approximately 15 ml of SPC agar medium into plate.
22. Temperature of media should be warm to touch.
23. Mix sample and agar by rotating plate clockwise 5 times, counter clockwise 5 times and up and down and crosswise 5 times in the form of a cross. This should ensure adequate mixing and distribution of sample.
24. After the sample dilution is put onto the plate, addition of the agar should be done as soon as possible.
25. Turn plates agar side up when solidified and incubate 48±3 h at 35±0.5 °C.
26. Wash down bench top area with disinfectant-Wescodyne**.**

Calculation and reporting:

Use an aid such as the Quebec Colony Counter.

Counting of colonies on selected plates:

1. Compute the count by multiplying the count by the dilution factor. For example, if there were 130 colonies on a 1/10-dilution plate, the count would be recorded as 1300/ml.
2. If there were 0 colonies from all dilutions and the highest dilution plated was a cc - the count would be recorded as < 1/ml.
3. Report results to 2 significant figures: *e.g.* Report a count of 142 as 140, a count of 155 as 160 and a count of 35 as 35.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017. Method 9251A.

(VII. 3) Total Coliform in Non Potable Water

(Multiple Tube Fermentation Technique)

Principle:

The standard test for the coliform group is carried out by the multiple tube fermentation technique (lactose fermentation). The results of the examination of replicate tubes and dilutions are replotted in terms of the most probable number (MPN) of organisms present. This method is used for testing fresh water of the River Nile.

Equipment:

1. Sterilized glass bottles (1000 ml).
2. Sterilized pipettes and graduated cylinders.
3. Test tubes racks.
4. Sterilized test tubes with metal caps, metal foil covers and Durham tubes.
5. Incubator adjusted to 35±0.5 ºC.
6. Autoclave adjusted to 121 ºC for 15 minutes.
7. Draying oven.
8. Sensitive balance.
9. Refrigerator.

Reagents and materials:

1. Phosphate buffer solution prepared from:

Solution (1):

- A 34 gram portion of potassium dihydrogen phosphate is dissolved in 500 ml distilled water.
- Adjust the pH to 7.2, and complete the solution to 1000 ml with distilled water.

Solution (2):

- A 50 gram portion of magnesium chloride or sulfate is dissolved in 1000 ml distilled water.
- The buffer solution is prepared by adding 1.25 ml of solution (1) to 5 ml of solution (2) and completing the volume to 1000 ml with distilled water.
- Pour in each sterilized test tube 9 ml, cover the test tube with metal cover and sterilize by autoclaving at 121 ºC for 15 minutes.

2. Lauryl tryptose broth medium:

- Weigh 35.6 g of the media powder and disperse into 1 liter of deionized water.
- Allow to soak for 10 minutes, and then swirl to mix and heat gently to dissolve.
- Dispense into sterilized test tubes with inverted Durham tubes.
- Sterilize by autoclaving at 121 ºC for 15 minutes.

Sample handling (fresh water of River Nile water):

1. Collect the sample ina 1 liter sterilized bottle.
2. The sample should be collected from the middle of the selected area at a depth of 1-2 meter from the surface and the container should not be fully filled to allow aerobic bacteria togrow.

Procedure:

1. Arrange the fermentation tubes of lauryltryptose broth in rows of five tubes each in a test tube rack per dilution (10, 1.0, 0.1, 0.01, 0.001).
2. Inoculate10 ml of the sample on each tube of the 1st row of lauryltryptose broth (double strength).
3. Inoculate 1 ml of the sample on each tube of 2nd row of lauryltryptose broth (single strength).
4. Put one tube of buffer solution beside the last three rows.
5. Inoculate 1 ml of the sample in first buffer tube to become dilution of sample 1/10.
6. Take 1 ml from the buffer 1/10, and inoculate it in a second buffer solution to become dilution 1/100.
7. Take 1 ml from the buffer solution 1/100 then inoculate it in a third buffer solution to become dilution 1/1000.
8. Inoculate 1 ml of dilution 1/10 and inoculate in each fermentation tubes of lauryl tryptose broth in a third row.
9. Inoculate 1ml of dilution 1/100 and inoculate in each fermentation tubes of lauryl tryptose broth in a fourth row.
10. Inoculate 1 ml of dilution 1/1000 and inoculate in each fermentation tubes of lauryl tryptosebroth in a fifth row.
11. Incubate all tubes in incubator at 35±0.5 ºC for 48 hours.

Interpretation:

1. Heavy growth, gas and acid production in a lauryltryptose broth within 48 hour is considered a positive total coliforms reaction.
2. Failure to produce gas (with or no growth) reveals a negative total coliforms reaction.
3. The positive result is turbidity and gas production in the Durham tube due to lactose fermentation and production of lactic acid and gas.
4. Calculate the number of positive tubes of coliform bacteria from table of MPN, and then multiply it by the number of inverted middle dilution.

Confirmatory test:

1. Submit all presumptive fermentation tubes showing any amount of gas, heavy growth or acidity within 48 h of incubation to confirmed test.
2. Transferring 1 loop full from the positive lauryltryptose broth tube to brilliant green broth to be incubated at 35±0.5°C for 48 hours.

3. The positive result is production of gas in the Durham tube. Calculate the MPN number of the coliform bacteria from the MPN Table (VII. 2).

Table (VII.2) MPN index at 95% confidence

Combinations of positives	MPN index /100 ml	Confidence limits	
		Low	High
0-0-0	<1.8	--	6.8
0-0-1	1.8	0.090	6.8
0-1-0	1.8	0.090	6.9
0-1-1	3.6	0.70	10
0-2-0	3.7	0.70	10
0-2-1	5.5	1.8	15
0-3-0	5.6	1.8	15
1-0-0	2.0	0.10	10
1-0-1	4.0	0.70	10
1-0-2	6.0	1.8	15
1-1-0	4.0	0.71	12
1-1-1	6.1	1.8	15
1-1-2	8.1	3.4	22
1-2-0	6.1	1.8	15
1-2-1	8.2	3.4	22
1-3-0	8.3	3.4	22
1-3-1	10.0	3.5	22
1-4-0	10.0	3.5	22
2-0-0	4.5	0.79	15
2-0-1	6.8	1.8	15
2-0-2	9.1	3.4	22
2-1-0	6.8	1.8	17
2-1-1	9.2	3.4	22
2-1-2	12	4.1	26
2-2-0	9.3	3.4	22
2-2-1	12	4.1	26
2-2-2	14	5.9	36
2-3-0	12	4.1	26
2-3-1	14	5.9	36
2-4-0	15	5.9	36
3-0-0	7.8	2.1	22
3-0-1	11	3.5	23
3-0-2	13	5.6	35
3-1-0	11	3.5	26
3-1-1	14	5.6	36
3-1-2	17	6.0	36
3-2-0	14	5.7	36
3-2-1	17	6.8	40
3-2-2	20	6.8	40

3-3-0	17	6.8	40
3-3-1	21	6.8	40
3-3-2	24	9.8	70
3-4-0	21	6.8	40
3-4-1	24	9.8	70
3-5-0	25	9.8	70
4-0-0	13	4.1	35
4-0-1	17	5.9	36
4-0-2	21	6.8	40
4-0-3	25	9.8	70
4-1-0	17	6.0	40
4-1-1	21	6.8	42
4-1-2	26	9.8	70
4-1-3	31	10	70
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-2-2	32	10	70
4-2-3	38	14	100
4-3-0	27	9.9	70
4-3-1	33	10	70
4-3-2	39	14	100
4-4-0	34	14	100
4-4-1	40	14	100
4-4-2	47	15	120
4-5-0	41	14	100
4-5-1	48	15	120
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-0-3	58	22	150
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-1-3	84	34	220
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-2-3	120	36	250
5-2-4	150	58	400
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400
5-3-3	170	70	400
5-3-4	210	70	400
5-4-0	130	36	400
5-4-1	170	58	400

5-4-2	220	70	440
5-4-3	280	100	710
5-4-4	350	100	710
5-4-5	430	150	1100
5-5-0	240	70	710
5-5-1	350	100	1100
5-5-2	540	150	1700
5-5-3	920	220	2600
5-5-4	1600	400	4600
5-5-5	> 1600	700	--

Limits for various combinations of positive results when five tubes are used for dilution (10 ml ,1 .0 ml, 0.1 ml) are given in Table VII. 3.

Table (VII.3) Positive results with different tubes and dilutions

No	Volume combination, ml					MPN Index	Positives No / 100
	10 ml	1.0 ml	0.1 ml	0.01 ml	0.001 ml		
A	5	5	1	0	0	5-1-0	330
B	4	5	1	0	0	4-5-1	48
C	0	0	1	0	0	0-0-1	1.8
D	5	4	4	1	0	4-4-1	400
E	5	4	4	0	1	4-4-1	400
F	5	5	5	5	2	5-5-2	45000

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017. Method 9221B.

(VII. 4) Fecal Coliform

(Multiple Tube Fermentation Technique)

Principle:

A fecal coliforms is facultative anaerobic, rod shaped, gram-negative, and nonsporulating bacterium. Coliform bacteria generally originate in the intestines of warm blooded animals. Fecal coliform are capable of growth in the presence of bile salts or similar surface agents to produce acid and gas from lactose within 24 hours at 44.5±0.2 ºC.

Equipment:

1. Sterilized test tubes with metal caps, metal foil covers and Durham tubes.
2. Test tubes racks.
3. Metal loop, 3.5 mm-diameter.
4. Autoclave adjusted to 121 ºC for 15 minutes.
5. Incubator adjusted to 44.5±0.2 ºC.

Reagents:

(EC medium):

1. Add dehydrated ingredient to deionized water, mix thoroughly and heat to dissolve.
2. Dispense in fermentation test tubes, approximately 10 ml, with inverted Durham tubes previously sterilized by autoclaving at 121 ºC for 15 minutes.
3. The pH of the medium should be 6.9±0.2 after sterilization.

Procedure:

1. Gently shake the lauryltryptose fermentation tubes showing gas, heavy growth, or acidity with a sterile 3 - 3.5 mm-dim. Metal loop and transfer growth from each presumptive fermentation tube to EC broth tube.
2. Incubate inoculated broth tubes 44.5±0.5 ºC for 24 hours.

Interpretation:

1. Heavy growth, gas and acid production in EC broth within 24 h or less is considered a positive fecal coliform reaction.
2. Failure to produce gas (with or no growth) constitutes a negative fecal coliform reaction.
3. Calculate the number of positive tubes of fecal coliform bacteria from Table (VII. 2) then multiply it by the number of inverted middle dilution.

Reference:

"Standard Methods for Examination of Water and Wastewater", 23RD Edition, American Public Health Association (APHA), Washington 2017.Method 9221E.

(VII.5) Total Coliform in Potable Water

(Multiple Tube Fermentation Technique)

Procedure:

1. Inoculate 10 tubes of lauryl tryptose broth medium (double strength) with 10 ml portions from sample.
2. Incubate all tubes in an incubator at 35±0.5 ºC for 48 hours.

Interpretation:

1. Heavy growth, gas and acid production in alauryltryptose broth within 48 hour less is considered a positive total coliform reaction.
2. Failure to produce gas (with or no growth) constitutes a negative total coliform reaction.
3. The positive result is turbidity and gas production in the Durham tube due to lactose fermentation and production of lactic acid and gas.

Calculation:

Calculate the number of positive tubes of coliform bacteria from Table (VII. 4).

Table (VII.4) Number of positive tubes of coliform bacteria (MPN)

No. of tubes giving positive reaction out of 10 (10 ml each)	MPN Index/ 100 ml	95 % Confidence limits, exact	
		Lower	Upper
0	<1.1	-	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13
5	6.9	2.5	15
6	9.2	3.3	19
7	12	4.8	24
8	16	5.8	34
9	23	8.1	53
10	>23	13	-

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23RD Edition, American Public Health Association (APHA), Washington 2017.Method 9221B.

(VII.6) Fecal Coliform

(Multiple Tube Fermentation Technique)

EC Medium:

1. Add dehydrated ingredient media to deionized water, mix thoroughly, and heat to dissolve.
2. Dispense in fermentation test tubes approximate 10 ml with inverted Durham tubes and sterilize by autoclaving at 121 ºC for 15 minutes. The pH of the medium should be 6-9±0.2 after sterilization.

Procedure:

1. Gently shake the lauryltryptose fermentation tubes showing gas, heavy growth, or acidity with a sterile 3-3.5 mm-diameter metal loop and transfer growth from each presumptive fermentation tube to EC broth tube.
2. Incubate inoculated broth tubes 44.5±0.5 ºC for 24 hours.

Interpretation:

Heavy growth, gas and acid production in EC broth within 24 h or less is considered a positive fecal coliform reaction. Failure to produce gas (with or no growth) constitutes a negative fecal coliform reaction.

Calculation:

Table of (VII.5) Calculation of the number of positive tubes of fecalfrom (MPN)

No. of tubes giving positive reaction out of 10 (10 ml each)	MPN Index/ 100ml	95% Confidence limits (exact)	
		Lower	Upper
0	<1.1	-	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13
5	6.9	2.5	15
6	9.2	3.3	19
7	12	4.8	24
8	16	5.8	34
9	23	8.1	53
10	>23	13	-

Reference:

"Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017. Method 9221 E.

(VII.7) Feacal Streptococcus Faeacalis

(Materials and Culture Media)

Azide dextrose broth:

1. Beef extract ……. 4.5 g
2. Tryptone or polypeptide …….. 15 g
3. Glucose…… 7.5 g
4. Sodium chloride ……..… 7.5 g
5. Sodium azide…… 2 g
6. Reagent grade water ……... 1 liter
7. pH should be 7.2±0.2 at 25 °C after sterilization.

Bile esculin azide agar:

1. 1.Yeast extract..... 5.0 g
2. 2.Proteose peptone No. 3.....3.0 g
3. 3.Tryptone .....17.0 g
4. 4.Oxgall .....10.0 g
5. 5.Esculin .....1.0 g
6. 6.Ferric ammonium citrate.....0.5 g
7. 7.Sodium chloride .....5.0 g
8. 8.Sodium azide.....0.15 g
9. 9.Agar.....15.0 g
10. 10.Reagent-grade water .....1 l

The pH should be 7.1±0.2 at 25°C after sterilization. Hold medium for not more than 4 h at 45 to 50 °C before plates are poured.

Procedure:

1. Inoculate 10 tubes ofAzide dextrosebroth medium (double strength) with 10 ml portions from sample.
2. Incubate all tubes in incubator at 35±0.5 ºC for 48 hours. Examine each tube for turbidity at the end of 48±3 h.

Confirmed test procedure:

1. Subject all azide dextrose broth tubes showing turbidity. Streak a portion of growth from each positive azide dextrose broth tubes on PSE agar. Incubate the inverted dish at 35±0.5 ºC for 24 hours.
2. Brownish-black colonies with brown halos confirm the presence of fecal streptococci.

Calculation:

Table of (VII. 6) Number of positive tubes (MPN) of fecal streptococci

No. of tubes giving positive reaction out of 10 (10 ml each)	MPN Index / 100 ml	95 % Confidence limits (exact)	
		Lower	Upper
0	<1.1	-	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017.Method 9230.

(VII.8) Total Bacterial Count

(Spread Plate Method)

Plate count agar media:

Typical formula	g/liter
Tryptone …….	5.0
Yeast extract ……	2.5
Glucose …….	1.0
Agar …….	9.0
Reagent – grade water ……	1 liter

1. The pH of the media should be 7.0±0.2 at 25°C after autoclaving at 121 ºC for 15 minutes.
2. Allow soaking for 10 minutes, swirl to mix then sterilize by autoclaving at 121ºC for 15 minutes. Cool to 47 ºC then pour into Petri dishes 10 ml.

Procedure:

Put 1 ml of the test sample in two Petri dishes then pour in plate count agar. Incubate one plate at 37 °C for 24 hours and incubate the other plate at 35 °C for 48±3 hours.

Counting and recording:

Count all colonies on the selected plates by using Quebec colony counter or an automatic plate counting instrument.

$$CFU/ml = \frac{\text{Colonies counted}}{\text{Actual volume of sample in the dish, ml}}$$

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017.Method 9215C.

(VIII) Biological Examination

(VIII.1) Phytoplankton Counting

(Microscopic Examination)

Principle:

Phytoplankton (microscopic algae) occurs as unicellular, colonial, or filamentous form. They are used as indicators of water quality, some species flourish in highly eutrophic water while others are very sensitive to organic or chemical waste. Some species develop noxious blooms, sometimes creating offensive tastes and odors or anoxic or toxic conditions resulting in animal deaths or human illness.

Sample collection:

- In streams and rivers, locate stations upstream and downstream from suspected pollution sources.
- Collect a sample volume of 0.5 to 1 liter in a labelled bottle with sufficient information such as date, sampling station, type of sample and depth.
- Concentration of sample by sedimentation from the initial container to sequentially smaller ones.

Concentration technique:

Concentration of sample by sedimentation from the initial container to sequentially smaller ones.

Counting technique:

From the well mixed concentrated sample, 1.0 ml was drowned and placed into a Sedgwick- Rafter cell. The cell was microscopically examined using readjusted eye piece and objective lens of the order (X 10) for the eye piece and a 20-mm objective. Counting was conducted on 50-fields of the cell using a calibrated micrometer adjusted to the eye piece.

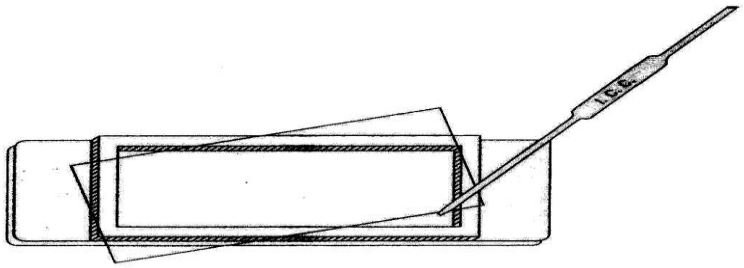


Fig. (VIII.1) Counting cell (Sedgwick- Rafter), showing method of filling.

To calculate the number of phytoplankton organisms present in a sample under investigation the following formula is applied:

$$\text{Number of phytoplankton/ml} = \frac{C \times At}{L \times W \times S \times V}$$

where:

C = number of organisms counted.

At = total area of bottom of setting chamber, mm^2 (Sedgwick- Rafter).
L = length of a strip, mm.
W = width of a strip, mm.
S = number of strips counted.
V = volume of sample settled.

Reference:

- "Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method 10200F.

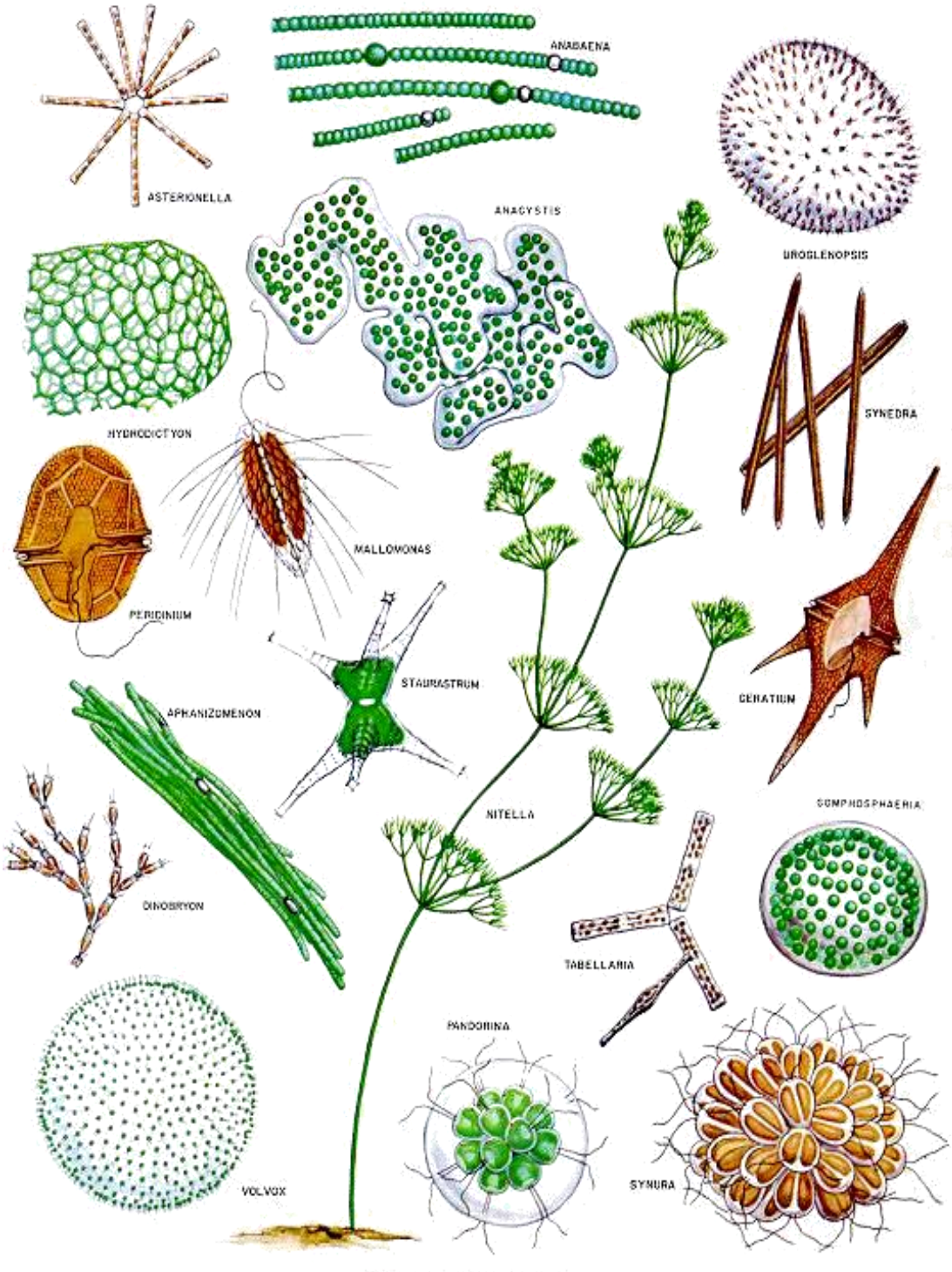


Fig.(VIII.2) Taste and odor algae.

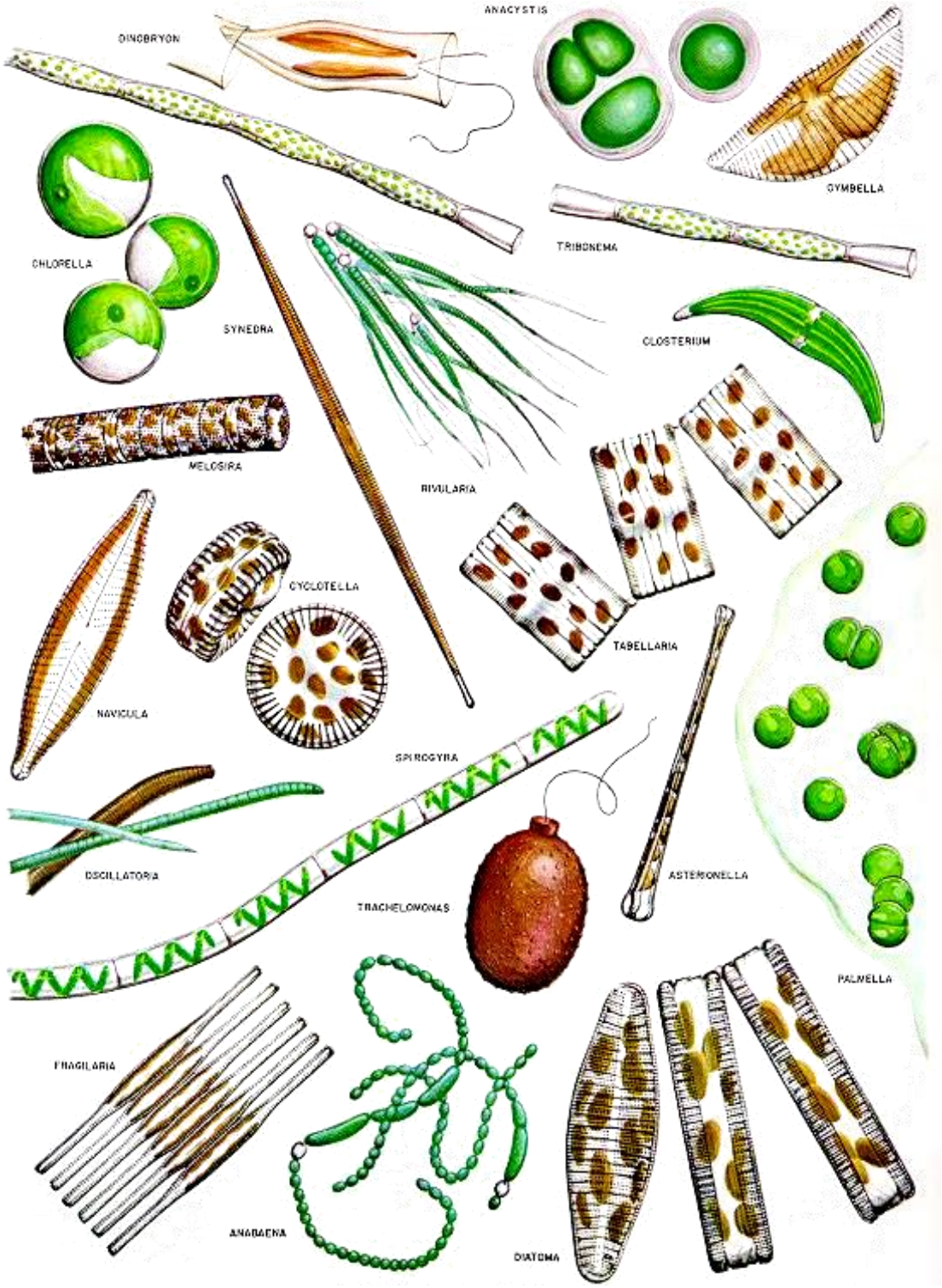


Fig. (VIII.3) Filter and screen- clogging algae.

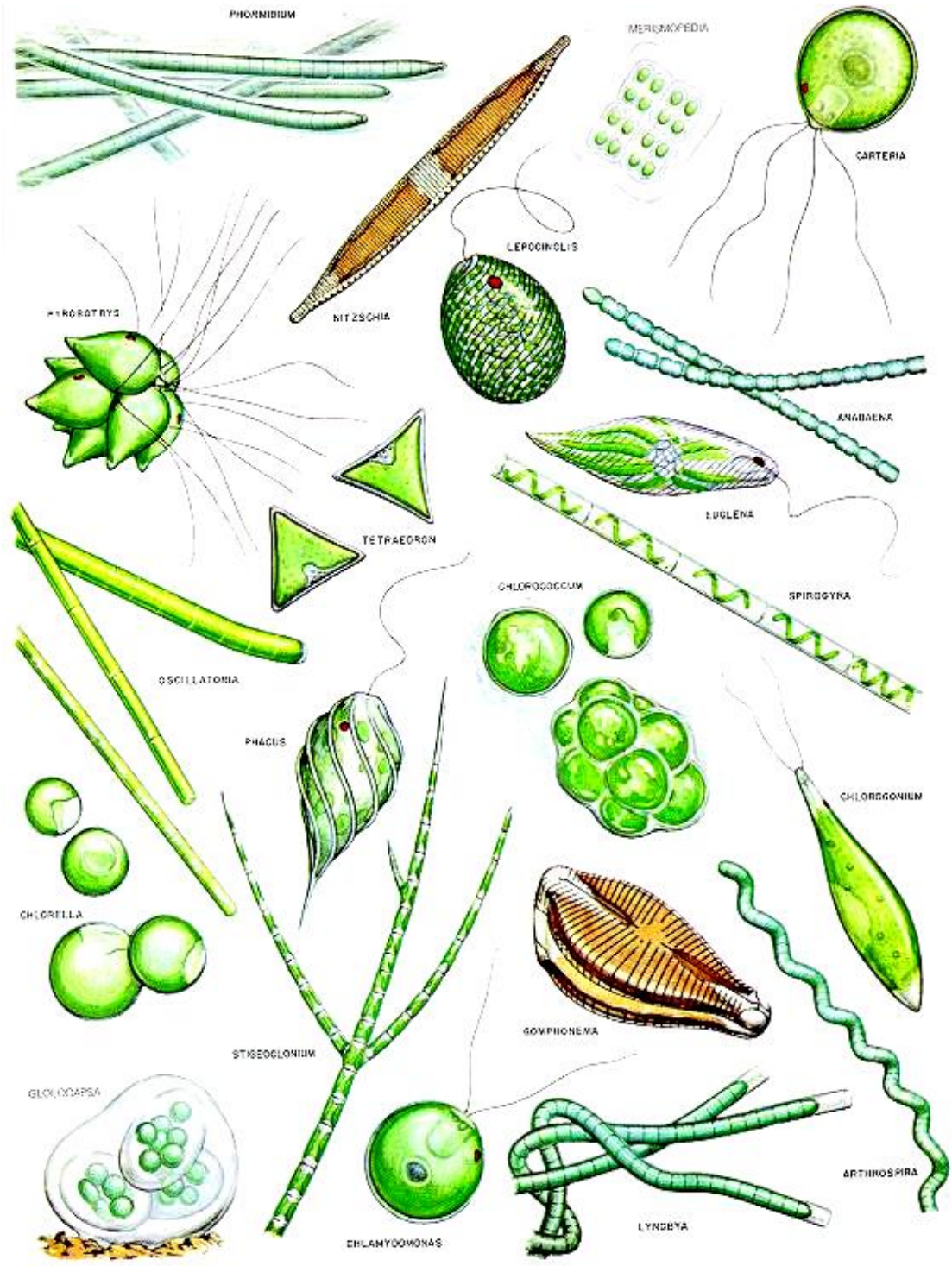


Fig. (VIII. 4) Fresh water pollution algae.

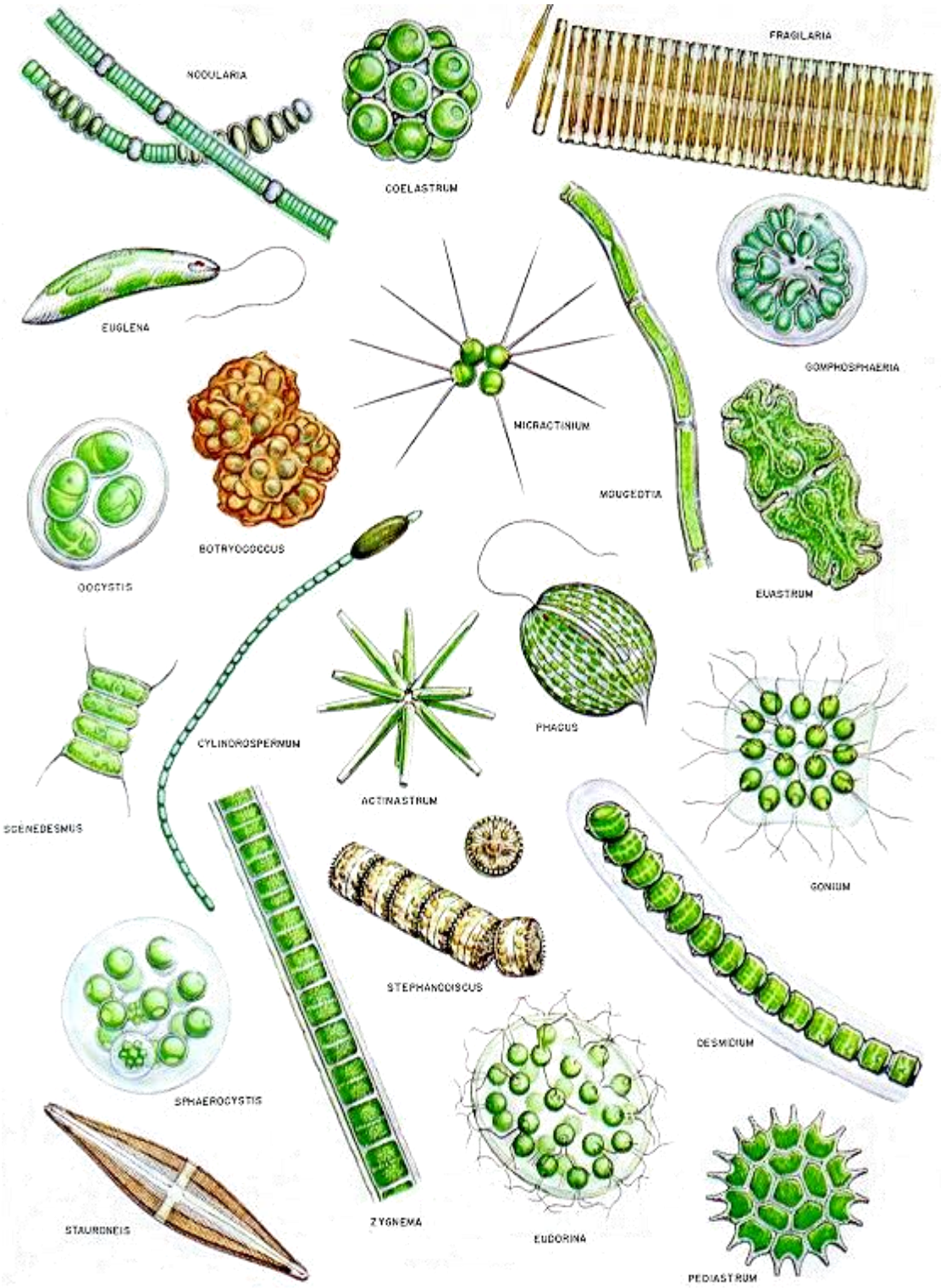


Fig. (VIII. 5) Plankton and other surface water algae.

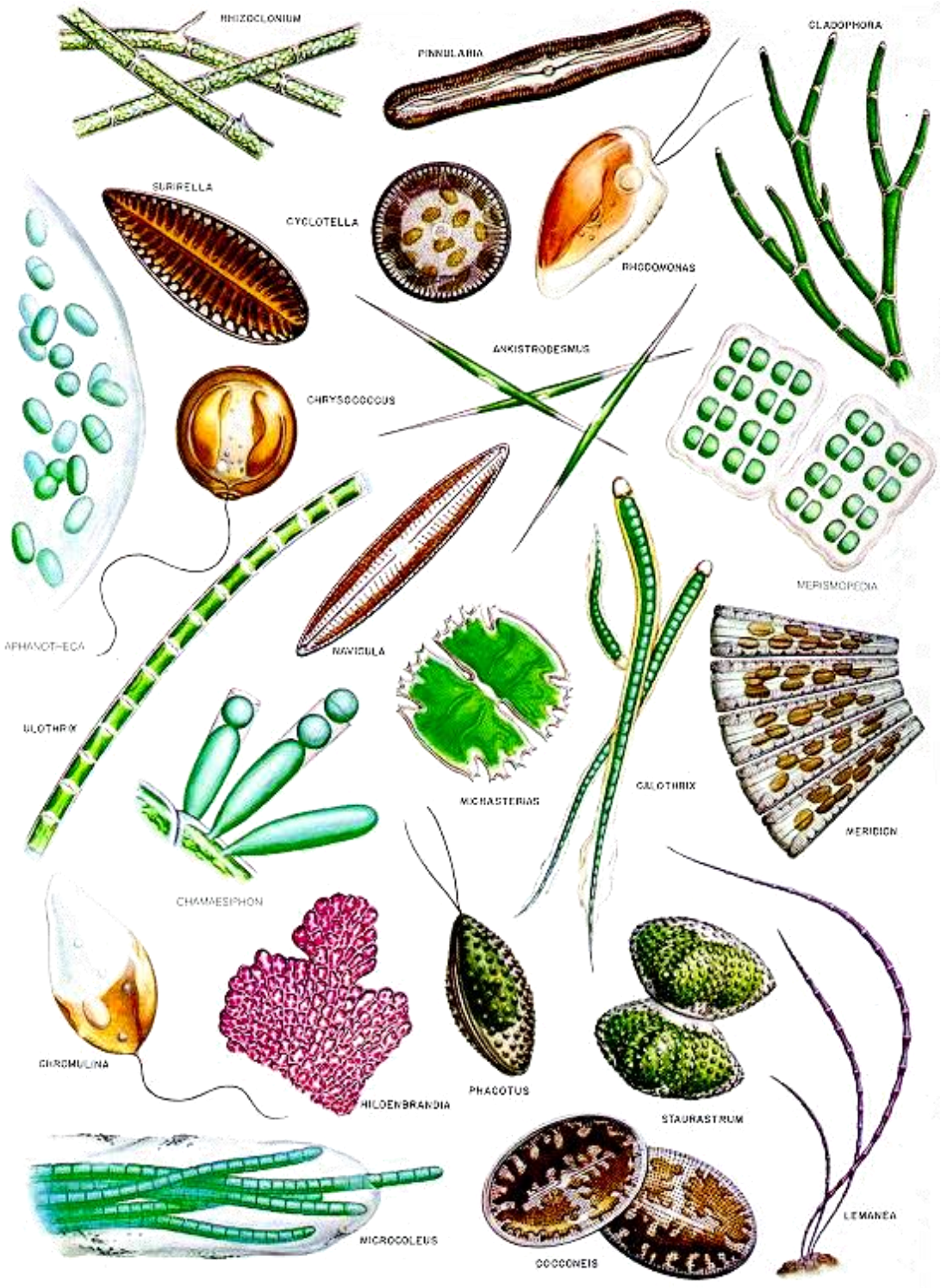


Fig.(VIII.6) Clean water algae.

(VIII.2) Enumeration of Intestinal Helminthes Eggs
(Microscopic Examination)

Principle:

The development of medical parasitology has led to a wide range of techniques for the enumeration of intestinal helminthes eggs. The parasites are floated away from other debris in a solution and be detected. The sample size must be 10 liter. Figure. (VIII.7) shows some different types of enteric nematode eggs.

Equipment:

1. Plastic containers for sample collection.
2. A centrifuge (capable of generation 1000 g).
3. Centrifuge tubes with lids tubes (50-ml and 15-ml tubes).
4. Pasteur pipettes and teats.
5. Mc Master counting slides (1 or 2).
6. A vortex mixer.
7. Graduated pipette.

Reagents:

1. Zinc sulfate solution (33 %, relative density 1.18).
2. Ether (or ethyl acetate).
3. Aceto-acetic buffer (pH 4.5): Dissolve 15 g sodium acetate trihydrate.
4. Glacial acetic acid (6 ml) diluted to 1 liter with distilled water.
5. Detergent solution (1ml Triton x-100 or Tween 80 made up to 1 liter with tap water).

Procedure:

1. Collect a sample of waste water of a known volume (v) (ca.10 ml) in a plastic container.
2. Allow the suspended materials in the sample to settle down as a sediment for 1 - 2 hours.
3. Remove 90 % of the supernatant using a siphon.
4. Transfer the sediment to one or more centrifuge tubes and centrifuge at1000 g for 15 min.
5. Rinse the container with the detergent solution and add the risings to the sediment.
6. Remove the supernatant.
7. Suspend the sediment pellet in an equal volume of acetoacetic buffer of pH 4.5.
8. Add two volumes of ethyl acetate and mix the solution thoroughly in a vortex mixer.
9. Centrifuge the sample in 1000 g for 15 min.

10. The sample will now have separated into three distinct phases. All the non – fatty, heavier debris, including helminthes eggs, larvae and protozoa, will be in the bottom layer. Above this layer, will be the buffer, which should be clear.

11. The fatty and other material move into the ethyl acetate.

12. Record the volume of the pellet containing the eggs, and then pour off the rest of the supernatant in one smooth action.

13. Resuspend the pellet in five volumes of zinc sulfate solution, record the volume of the final solution (x ml) and mix the sample thoroughly by using vortex mixer.

14. Transfer 0.15 ml of the final product with a Pasteur pipette in each chamber (P) to fill a McMaster slide for examination under the microscope 10 x or 40 x.

15. Count the eggs seen (A).

16. For greater accuracy, the mean of two or threeslides, should be recorded.

Calculate the number of eggs (N) per liter from the equation:

$$N = \frac{AX}{PV}$$

Where**:**

N = number of eggs per liter of sample.

A = number of eggs counted in a McMaster slide or the mean of counts from two or three slides.

X = volume of the final product (ml).

P = volume of the McMaster slide (0.3 ml).

V= original sample volume (liter).

Reference:

- World Health Organization, Geneva, 1996.

Ascaris Lumbercoides

Trichuris Trichiura

Taenia saginata & Solium

Enterobius Vermicularis

Hymenolypis Nana

Fig. (VIII.7) Different types of enteric nematode eggs.

(IX) Radioactivity Measurements

(IX.1) Gross Alpha and Gross Beta

(Radiometry)

Principle:

The thin-window, heavily shielded, gas-flow, anti coincidence circuitry proportional counter is the recommended instrument for counting gross alpha and beta radioactivity because of its superior operating characteristics. These include a very low background and a high sensitivity to detect and count an alpha and beta radiation range that is reasonable but not as wide as that of internal proportional counters. The instrument is calibrated by adding portions of standard nuclide to media comparable to water test samples and preparing, mounting, and counting the standards exactly as the samples. An internal proportional or Geiger counter also may be used; however, the internal proportional counter has a higher background for beta counting than the thin-window counter. Alpha activity cannot be determined separately with a Geiger counter but can be measured with either a thin-window or internal proportional counter; counting efficiency is higher for the internal counter. For testing water samples, an appropriate volume of water is evaporated and the residue is subjected to radiation examination.

Equipment:

1. Counting pans, of metal resistant to corrosion from solid sample or reagents, about 50 mm diam, 6 to 10 mm in height, and thick enough to be serviceable for one-time use. Stainless steel planchets are recommended for acidified samples.
2. Thin end-window proportional counter, capable of accommodating a counting pan. Alternate counters (internal proportional and Geiger counters).
3. Membrane filter, 0.45-µm pore diameter.
4. Gooch crucibles.
5. Counting gas, as recommended by the instrument manufacturer.

Reagents:

1. Methyl orange indicator solution.
2. Nitric acid, HNO_3, 1 N.
3. Clear acrylic solution: Dissolve 50 mg clear acrylic in 100 ml acetone.
4. Ethyl alcohol, 95 %.
5. Conducting fluid: Prepare according to manufacturer's directions (for internal counters).
6. Standard certified thorium-230, cesium-137, or strontium- 90, yttrium-90 solution.
7. Standard certified americium-241, plutonium-239, or natural uranium solution. For natural uranium, use material in secular equilibrium.
8. Reagents for wet-combustion procedure:

- Nitric acid, HNO_3, 6 N.
- Hydrogen peroxide solution: Dilute 30 % H_2O_2 with an equal volume of water.

Procedure:

1. For each 20 cm^2 of counting pan area, take a volume of sample containing not more than 200 mg residue for beta examination and not more than 100 mg residue for alpha examination. The specific conductance test on a non preserved sample helps to select the appropriate sample volume.
2. Evaporate by either of the following techniques:
   a. Add sample directly to a tared counting pan in small increments, with evaporation at just below boiling temperature. This procedure is not recommended for large samples.
   b. Place sample in a borosilicate glass beaker or evaporating dish, add a few drops of methyl orange indicator solution, add 1 N HNO_3 dropwise to pH 4 to 6, and evaporate on a hot plate or steam bath to near dryness. Avoid baking solids on evaporation vessel. Transfer to a tared counting pan with the aid of a rubber policeman and distilled water from a wash bottle. Using a rubber policeman, thoroughly wet walls of evaporating vessel with a few drops of acid and transfer washings to counting pan. Excess alkalinity or mineral acidity is corrosive to aluminum counting pans.
3. Complete drying in an oven at 103 to 105 °C, cool in a desiccator, weigh, and keep dry until counted.
4. Treat sample residues having particles that tend to be airborne with a few drops of clear acrylic solution, then air- and oven-dry and weigh.
5. With a thin end-window counter count alpha and/or beta activity.
6. Store sample in a desiccator and count for decay if necessary. Radionuclides that are volatile under the sample preparation conditions of this method will not be measured. In some geographic areas nitrated water solids (sample evaporated with nitric acid present) will not remain at a constant weight after being dried at 105 °C for 2 h and then exposed to the atmosphere before and during counting. Other radioactive substances (such as some chemical forms of radioiodine) also may be lost during sample evaporation and drying.
7. Heat such samples to a dull red heat for a few minutes to convert the salts to oxides. Sample weights then usually are sufficiently stable to give consistent counting rates and a correct counting efficiency can be assigned. Radioisotopes such as those of cesium may be lost when samples are heated to dull red color. Such losses are limitations of the test method.

Calculation:

A. Alpha activity:

Calculate alpha activity, in picocuries per liter, by the equation:

$$\text{Alpha} = \text{net cpm} \times 1000 / 2.22 \text{ ev}$$

where:

e : calibrated overall counter efficiency .

v: volume of sample counted, ml.

B. Beta activity:

Calculate and report gross beta activity and counting error in picocuries per liter of fluid, per kilogram of moist (live weight) biological material, or per kilogram of moist and per kilogram of dry silt.

If significant alpha activity is indicated by the sample alpha plateau count, determine beta activity by counting the sample at the beta plateau and calculating:

$$\text{Beta, pCi/l} = \beta - \alpha M / 2.22 \times D \times V$$

where:

β = net beta counts at the beta plateau.

α = net alpha counts at the alpha plateau.

M = alpha amplification factor (from ratio plot), 2.22 = dpm/pCi.

D = beta counting efficiency, cpm/dpm.

V =: sample volume, liter.

Reference:

-"Standard Methods for Examination of Water and Wastewater", 23rd Edition, American Public Health Association (APHA), Washington 2017, Method 7110 B.

(X) Quality Control (QC) and Quality Assurance (QA)

1. Quality assurance plan:

Quality assurance (QA) refers to the full range of practices employed to ensure that laboratory results are reliable. The term encompasses internal and external quality control. A sound water quality sampling and analysis program is commonly including a detailed Quality Assurance/Quality Control Plan that describes:

- Data quality objectives.
- Equipment maintenance and calibration.
- Chain of custody for samples.
- Quality control checks.
- Data reduction, validation, and reporting.

Objectives:

Monitoring data of drinking water are used to support a number of activities including compliance with the law; water management; reporting the quality and quantity of various pollutants in the drinking water. Sampling ranging from daily to quarterly at fixed locations for various durations of time (weeks, months, years) are collected and analyzed for some parameters. The monitoring program is focused primarily on conventional parameters such as pH, dissolved and suspended solids, residual chlorine, and bacteriology. Other parameters susceptible to change due to anthropogenic sources are also measured such as nutrients (total phosphorus and oil and grease. Phosphate-phosphorus, nitrates, nitrites and ammonia as well as chemical and biochemical oxygen demand. The role of the drinking water monitoring program is to provide timely water quality data and periodic data analysis reports, and to make these data and reports available to other potential users (governmental agencies).

1. Sampling design:

- Grab and/or composite samples are collected at the selected locations and frequency as indicated in this manual. This sampling frequency was chosen in order to optimize the probability of statistically detecting trends. Sample collection generally occurs at a set time each month.
- All samples requiring laboratory analyses are placed in the containers provided by the lab and labeled with the date, sample site, sample identification number (previously assigned by the lab for each sample), sampler's initials, and the chemical analyses requested. Preservatives, if required, are typically added to the bottle by the lab prior to sampling. Samples are then placed on ice and delivered to the lab according to procedures prearranged with the lab. Sampling equipment is rinsed thoroughly with de-ionized water after processing samples.

2. Laboratory procedures:

Laboratory analyses and laboratory procedures are following Standard Operating Procedures and other guidance documents.

3. Quality control program:

Standard Operating Procedures (SOP's) for the analysis of each parameter and their Quality Assurance Manual and QC program includes the analysis of reference materials, check standards, duplicates, matrix spikes, control chart and blanks.

4. Check standards:

Precision is addressed by the analysis of check standards (water with a known concentration of analyte) equal to about 10 % of the total number of analyses. The mean value for a statistically significant number of check standard results may be used to judge whether there is any bias due to calibration. If the 95 % confidence limit on the mean value does not include the true or reference value then bias due to calibration may be present. Generally, calibration standards are set by MEL as needed to bracket the concentration in particular samples. The check standards should equitably span the range of the expected results, ideally approximately 0.2 and 0.9 of the upper value for the range of calibration.

5. Duplicate samples:

These are simply two identical samples collected and handled in the same way they measure the precision of your methods.

A. Location duplicates: These are two samples collected from the same location at the same time; these measure the precision of your entire procedure (sampling, storage and handling, and laboratory analysis).

B. Laboratory duplicates: These are two samples split from a single sample once it has arrived at the laboratory. These test the precision of the laboratory methods only.

6. Matrix spikes:

Matrix interference leading to bias is assessed by analyzing a wastewater sample that has been spiked with a known quantity of the analyte. The quantity of analyte added should not produce a final concentration that is excessively high when compared to the highest range of data. Spike amounts should approximately double the concentration in the sample prior to spiking.

7. Recovery (accuracy):

Recovery is defined as the 'fraction of the analyte determined after addition of a known amount of the standard analyte to a drinking water sample'. In practice, control samples are most commonly used for spiking. The samples as well as the spikes are analyzed at least 10 times, the results averaged and the relative standard deviation (RSD) calculated. The recovery is calculated using the equation:

$$\text{Recovery, \%} = [(X_s – X)/ X_{add}] \times 100$$

where :

X_s = mean result of spiked drinking water samples.

X = mean result of unspiked drinking water samples.

X_{add}= amount of added standard analyte.

8. Blanks:

Blanks are samples containing pure, uncontaminated water. Blanks contain none of the measured parameters and are used to identify contamination that might occur during sampling or in the laboratory.

A. Location blank: This is a blank sample that is placed in a sample bottle at the sampling site, and is handled the same as a normal sample. It identifies contamination that might occur in the entire procedure (from field sampling to laboratory analysis).

B. Laboratory blank: This is prepared at the laboratory and tests for laboratory contamination only.

9. Trueness:

This is expressed by the equation:

$$\text{Trueness, \%} = (X / \mu) \times 100$$

where:

X= mean of test results obtained for reference sample.

μ = "true" value given for reference sample.

10. Bias:

It is more commonly used than trueness, and expressed by the equation:

$$\text{Bias, \%} = [(X – \mu) / \mu] \times 100$$

11. Precision:

Replicate analyses performed on a reference sample can be used to determine trueness or bias, as described above, as well as a standard deviation of the mean as a measure for precision. However, for precision alone also control samples and even test wastewater samples can be used. Numerically, precision is expressed by the relative standard deviation (RSD) or coefficient of variation (CV).

$$\text{Precision, \%} = (S/X) \times 100$$

Where:

X = mean of test results obtained for the reference standard sample.

S = standard deviation of x.

$S^2 = \sum (X_1 – X)^2 / n-1$.

n = degree of freedom.

12.Reproducibility:

This is a measure of the spread of results when a sample is analyzed by different laboratories. This is a measure of agreement between results obtained with the same method on identical test or reference material under different conditions (execution by different persons, in different laboratories, with different equipment and at different times). The measure of reproducibility R is the standard deviation of these results s_R, and for a not too small number of data ($n < 8$) R is defined by (with 95 % confidence):

$$R = 2.8 \times s_R$$

(Where $2.8 = 2\sqrt{2}$ and is derived from the normal or Gaussian distribution (ISO 5725).

13. Repeatability:

The measure of agreement between results obtained with the same method on identical test or reference standard sample under the same conditions (job done by one person, in the same laboratory, with the same equipment, at the same time or with only a short time interval). Thus, this is the best precision a laboratory can obtain: the within-batch precision. The measure for the repeatability r is the standard deviation of these results s_r, and for a not too small number of data ($n > 10$) r is defined by (with 95 % confidence):

$$r = 2.8 \times s_r$$

14. Control charts:

Control charts are used for recording internal quality control data .The principle of control charts is that IQC data can be graphically plotted so that they can be readily compared and interpreted. Various types of control charts can be used such as X-chart and R-chart. X-chart is a graph with time (or assay batch) on the x-axis and the concentration of the variable in the reference material on the y axis. The mean of a number of control values obtained over a suitably long period of time is used as the central line in the chart. Two other lines above and two below the central line are also drawn. These are the upper and lower warning limits and the upper and lower action limits. The limits are based on two and three times the standard deviation of the batch means, respectively. Provided the distribution is normal, 95 per cent of results from assays in control will fall between the two warning lines. Action lines are normally placed at three standard deviations to either side of the target line and 99 per cent of normally distributed results should be between the action lines. Examples of typical X- charts are shown below.

An R-chart is a similar control chart in which the mean range of repeated measurement is used as the central line, the control values being the difference between highest and lowest response value for a control sample in one batch. R-charts are normally used only with action limits. In the regular day-to-day use of the control charts, an aliquot from an appropriate reference material is analyzed with every batch of samples and the measured concentration of the variable in the aliquot is plotted on the chart.

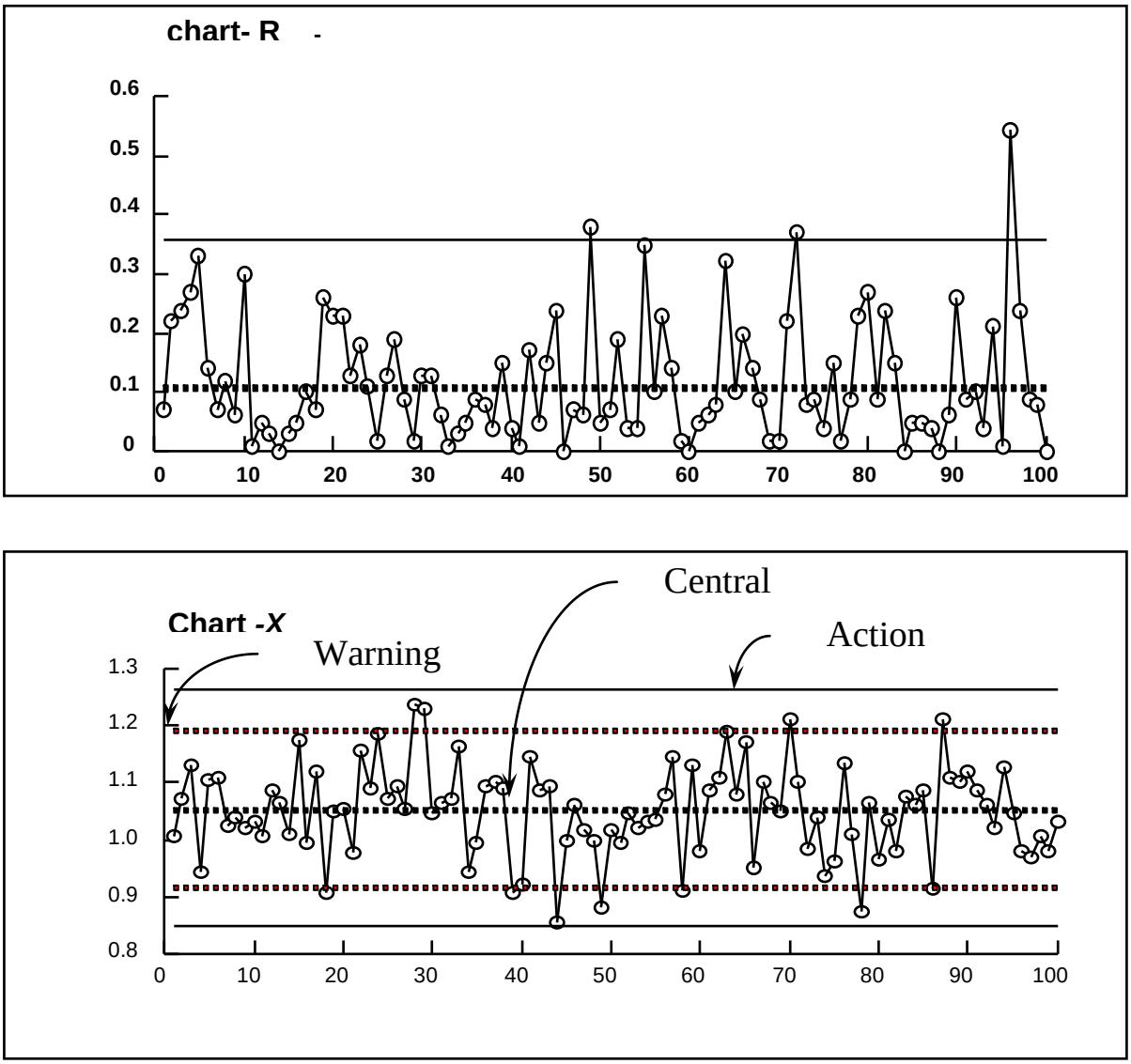


Fig. (X.1) Typical R- and X-charts.

Out-of control situation exists when; 1 control value outside the action limit, 2 consecutive control values outside the warning limits, 7 consecutive values with raising or failing tendency. In addition, 10 out 11 consecutive control values on one side of the central line in case of X-chart and 7 consecutive values above the central line in R-charts are also considered as out- of –control (ISO 13530).

Out-of control situation requires prompt detailed checking of the analytical method and rejection of the assay data. Results outside the action limits should prompt detailed checking of the analytical method and rejection of the assay data.

15. Lower limit of detection:

It is the concentration of the analyte giving a signal equal to the blank plus 3X the standard deviation of the blank. Because in the calculation of analytical results the value of the blank is subtracted (or the blank is forced to zero) the detection limit can be written as:

$$LLD, MDL = 3 \times s_{bl}$$

At this limit it is 93 % certain that the signal is not due to the blank but that the method has detected the presence of the analyte (this does not mean that below this limit the analyte is absent).

16.Instrumentation:

The pH meter and spectrophotometer are calibrated according to the manufacturer's directions. The pH meter is checked immediately after calibration, at mid-day, and at the end of the day by recording the measurement at a low ionic strength pH 7 buffers. It is also checked whenever a measurement exceeds the quality standards criteria. If the difference between the meter measurement and the expected pH exceeds 0.10, the instrument is recalibrated and the sample re-measured. The spectrophotometer is relatively stable; the calibration is generally checked at the beginning of wastewater sample measurements.

17. Corrective action:

An appropriate series of corrective actions can be taken to keep the quality control, these are:

- Review quality control limit calculations for obvious errors.
- Rest the data for outliers, exclude any that are identified as such, and re-calculate control limits.
- Review matrix spike preparation procedures to determine if any errors were made.

The laboratory continually monitors their results for quality control sample determinations and takes appropriate action to correct problems. Frequently, samples may be re-analyzed after an analytical problem is corrected. This is also the case for location measurements with respect to check standard results. Due to sample holding time limitations, re-analysis is usually not possible if problems are discovered in field QC data. Corrective courses applying to subsequent data collection are possible, however.

If data are compromised due to poor precision, the source of the variability will determine the course of action that is required. Possible actions include:

a. Changing the standard operating procedures or instrumentation.
b. Informing the laboratory when lab error appears to be the source (and possibly changing analytical methods).
c. Re-evaluating the required precision, when it appears that the required error is unattainable.

A persistent, consistent bias in the data may warrant adjusting the values, otherwise the corrective action for bias will be to inform the lab, which will be expected to address the problem. Significant changes in methods, instrumentation, or protocols will be made only after it has been documented that these changes will not bias the data.

Summary of an internal quality control program:

For each parameter:

a. Analyze five standard solutions at six different known concentrations covering the working range to develop a calibration curve or, when a calibration curve already exists, analyze two standard solutions at different known concentrations covering the working range to validate the existing calibration curve.
b. Analyze one method blank per set of 20 drinking water samples.
c. Analyze one field blank per set of drinking water samples.
d. Analyze one duplicate of a drinking water sample chosen at random from each set of up to 20 samples.
e. Analyze one drinking water sample that has been spiked with a known amount of the variable as a recovery check. This sample should have a matrix similar to those of the drinking water samples being processed.

18. Data management procedures:

The data are managed first by recording data for parameters measured in the field manually on a standard form and entered by the sampler into a temporary computer access table upon return to the laboratory. Rough validation rules prohibit obviously incorrect data from being entered. A hardcopy of the temporary table is printed and the sampler reviews the data prior to moving it into the final results table. After field and laboratory data are entered, an evaluation of results is performed.

19. Reports:

These reports are identified as being based on preliminary data. After a full month's data are available, all results are reported and all results exceeding water quality criteria or the usual range of results from a particular location are identified. Upon completion of the data collection activities, the previous year's program is summarized in an annual report. This report includes an analysis of field and some lab QC data collected during the year as well as an appendix listing known changes to the monitoring program that could potentially affect the data.

20.Data review, verification, and validation:

Data verification prior to reporting includes an on-going evaluation of their QC results (using control charts, etc consists of a computer assessment of the data and associated field QC data):

- Each result is compared to historic data from that collection location during the same season. The datum is 'flagged' if it lays more than 2.5 standard deviations from the mean.
- The values of replicated samples are flagged if the coefficient of variation of the replicates or split samples exceeds 20 %.
- The datum is flagged if the holding time was exceeded.
- If internal logic checks (total phosphorus greater than soluble reactive phosphorus or total nitrogen greater than nitrate/nitrite plus ammonia) are violated, then all data values involved are flagged.

21.Data quality assessment:

Result-level data validation procedures are conducted monthly as described. QA assessments is made by comparing calculated percent of relative standard deviations (% RSD) to those specified in MQOs:

$$\% \text{ RSD} = 100 \text{ x } (S/X) = \quad 100 \text{ x } \sqrt{[(r_1 - r_2)^2 /2 \,/\, (r_1 - r_2)/2]}$$

Where S is the standard deviation, X is the mean, and r_1 and r_2 are paired results, typically a known value (e.g., of a check standard) and the analytical result or measurement of the known value.

The results of the analysis of blank samples and known standards will be used to determine overall bias of the results.

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