Experiment #1A

pH MEASUREMENT

1. Objective:

The purpose of this laboratory activity is to understand the theory of pH measurement and measure the pH of water sample with a pHmeter.

2. Theory:

pH is a measure of hydrogen ion concentration in aqueous solution. It is an important parameter to determine the quality of water. The pH value is expressed as:

$$pH = 1 / (\log_{10} C)$$

Where C is the concentration of H^+ ions in a solution. In pure water, the concentration of H^+ ions is 10^{-7} *g/l* at 25^{o} C.

So the pH value is

$$pH = 1 / (\log_{10} 10^{-7}) = 7$$

The pH value of a solution is measured by using pH electrode. It essentially consists of a pair of electrodes: *measuring* and *reference* electrode, both dipped in the solution of unknown pH. These two electrodes essentially form two *half-cells;* the total potential developed is the difference between the individual electric potential developed in each half cell. While the potential developed in the reference cell is constant, the measuring cell potential is dependent on the hydrogen ion concentration of the solution.

Measuring Electrode

The measuring electrode is made of *thin sodium ion selective glass*. A potential is developed across the two surfaces of this glass bulb, when dipped in aqueous solution. This potential is sensitive to the H^+ ion concentration. Fig. 1 shows the basic schematic of a measuring probe. The buffer solution inside the glass bulb has a constant H^+ ion concentration and provides electrical connection to the lead wire.

Reference Electrode

The basic purpose of a reference electrode is to provide continuity to the electrical circuit, since the potential across a single half cell cannot be measured. With both the measuring and reference cells dipped in the same solution, the potential is measured across the two lead wires. A reference electrode should satisfy the following basic requirements:

(i) The potential developed should be independent of H^+ ion concentration.

(ii) The potential developed should be independent of temperature

(iii) The potential developed should not change with time.

Considering all these requirements, two types of reference electrodes are commonly used: (i) Calomel (Mercury-Mercurous Chloride) and (ii) Silver-Silver Chloride. The construction of a Calomel reference electrode is shown in Fig. 2. The electrical connection is maintained through the *salt bridge.*

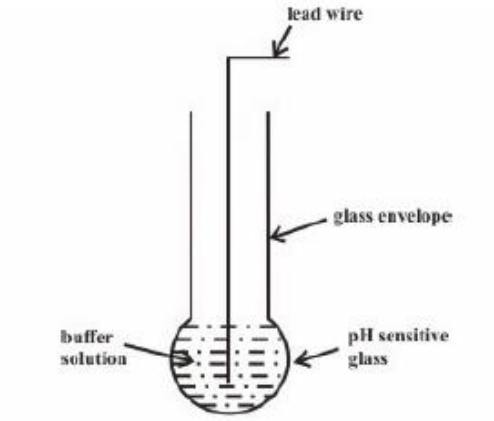


Fig 1. Basic schematic of a measuring probe

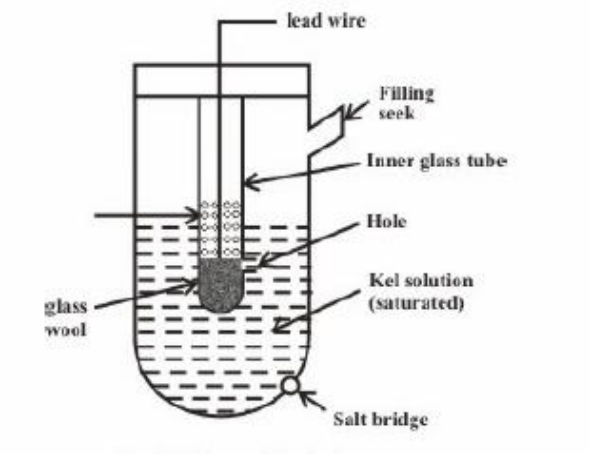


Fig 2. Basic schematic of a reference probe

3. Materials:

Apparatus:

-Beakers

-pHmeter

Chemicals:

-Calibration solutions

4. Procedure:

-pH measurement are taken with simply submerge the electrode tip (at least 4 cm / 1 ½") and the temperature probe into the test sample.

-pH mode is selected by pressing the appropriate key until the display changes to pH.

-Then we stir gently and wait for a symbol of stability (hourglass) to turn off. The pH value automatically compensated displayed for temperature.

Experiment #1B

MEASUREMENT OF ELECTRICAL CONDUCTIVITY

1. Objective:

The purpose of this laboratory activity is to understand the theory of electrical conductivity (EC) measurement and measure the EC of water sample with a conductivity meter.

2. Theory:

The conductance or conductivity expresses how well a material conducts an electric current. With metals, it is the movement of electrons that causes the current flow. In aqueous solutions, ions take over the charge transport. Ions are formed when salts, acids, or alkalis dissolve. The more ions are present in the liquid, the better it conducts the current.

This relationship between the ion concentration and the ability to conduct the electric current makes the conductivity an interesting process variable in water analysis. It is especially suited for determining the concentration of dissolved salts.

The result of a conductivity measurement is not quoted in mg/liter or percent, but as a conductivity value in S/m (siemens per meter). In practice, the smaller units µS/cm and mS/cm are commonly used.

Table 1. Conversion of conductivity units

	S/m	S/cm	mS/cm	µS/cm
S/m	1	0.01	10	10,000
S/cm	100	1	1,000	1,000,000
mS/cm	0.1	0.001	1	1,000
µS/cm	0.000 1	0.000 001	0,001	1

The following table illustrates the relationship between the salt concentration and the conductivity.

Table 2. Examples of conductivity values

Water or aqueous solution	Conductivity range at 25 °C	Salt concentration
High-purity water	0.055µS/cm	0mg/l
Fully-desalinated water	0.055 to 2µS/cm	0 to 1mg/l
Rainwater	10 to 50µS/cm	5 to 20mg/l
Ground, surface and drinking water	50 to 1000µS/cm	20 to 50mg/l
Sea water	20 to 60mS/cm	10 to 40g/l
Saline solution	77 to 250mS/cm	50 to 250g/l

The basic principle of conductivity measurement is the same with all methods: the instrument generates an electric voltage across the measured solution. An electric current flows whose value depends on the conductivity. Depending on the method or application, the instrument either maintains the voltage signal constant and records the change in electric current, or maintains the current value constant and evaluates the voltage change.

Both measurement principles are based on Ohm´s law:

$$R = \frac{U}{I} = \frac{1}{G} \quad (1)$$

R: electrical resistance
I: electrical current
U: electrical voltage, or rearranged for the conductance
G: conductivity

$$\gamma = \frac{I}{U} \bullet k' \quad (2)$$

γ: (specific) electrical conductance
I: electrical current
U: electrical voltage
k': cell constant

At constant voltage, the current increases proportionally with the conductance. At constant current, the voltage decreases with increasing conductance. It is clear from Ohm's law that conductivity measurements really concern resistance measurements. The conductance value I/U is obtained from the reciprocal of the resistance.

EC varies with temperature, and values reported are usually corrected to 25°C. Such data are known as Specific Conductance. A difference of 5°C can alter conductivity by approximately 10%. Many conductivity instruments have compensation functions so that EC at 25°C can be read directly

3. Materials:

Apparatus:

-Beakers

-Conductivity meter

4. Procedure:

-Wash the conductivity electrode with distilled water before using it; dry it so that it won't change the concentrations of samples by carrying either solid or water droplets.

-Immures the electrode in the beakers containing your water sample, note the measured conductivity with the units.

-Wash the electrode before using it for the other samples.

Experiment #2

DETERMINATION OF TURBIDITY

1. Objective:

To be familiar with the concepts of turbidity and the measurement of turbidity in water.

2. Theory:

Turbidity can be defined as the waters that contain suspended matter that interferes with the passage of light through the water or in which visual depth is restricted. Turbidity is a principal physical characteristic of water and may be caused by a wide variety of suspended materials, which range in size from colloidal to coarse dispersions, depending upon the degree of turbulence.

In general the materials that cause turbidity may range from purely inorganic substances to those that are largely organic in nature. This disparity in the nature of the material causing turbidity makes it impossible to establish hard and fast rules for removal.

Turbidity may be caused by a wide variety of materials.(Table 1). In glacier-fed rivers and lakes most of the turbidity is due colloidal rock particles produced by the grinding action of the glacier. As rivers descend from mountains area onto plains, they receive contributions of turbidity from farming and other operations that disturb the soil. Under flood conditions great amounts of topsoil are washed to receiving streams. Much of this material is inorganic in nature and includes clay and silt, but considerable amounts of organic matter is included. As the rivers progress towards the ocean, they pass through urban areas where domestic and industrial wastewaters, treated or untreated, may be added. The domestic waste may add great quantities of organic and some inorganic materials that contribute turbidity. Street washings contribute much inorganic and some organic turbidity. Organic materials reaching rivers serve as food for bacteria, and the resulting bacteria growth and other microorganisms that feed upon the bacteria produce additional turbidity. Inorganic nutrients such as nitrogen and phosphorous present in wastewater discharges and agricultural runoff stimulate the growth of algae, which also contribute turbidity.

Table 1. Sources of turbidity

Sources of Turbidity
Soil Erosion
Silt
Clay
Urban Runoff
Road grime
Rooftops
Parking lots
Industrial waste
Sewage treatment effluent
Particulates
Abundant bottom-dwellers
Stirring up sediments
Organics
Microorganisms
Decaying plant and animals
Gasoline or oil from roads

Turbidity is measured in many ways, but the main method to determine it, is by using a Nephlometer, Turbid Meter. Nephlometer measures the scattering light from particles with and uses the Nephlo Turbidity Unit (NTU). In the instrument, a light source illuminates the sample and one or more photoelectric detectors are used with a readout device to indicate the intensity of scattered light at right angles to the path of the incident light (Figure 1). Earlier SiO2 was used as a standard but now it is customary to use a particular Formazin polymer suspension as a standard. Samples with turbidities greater than max. NTU reading are diluted with turbidity free water until values within the range of instrument. The turbidity is then determined by multiplying the measured turbidity by the dilution factor.

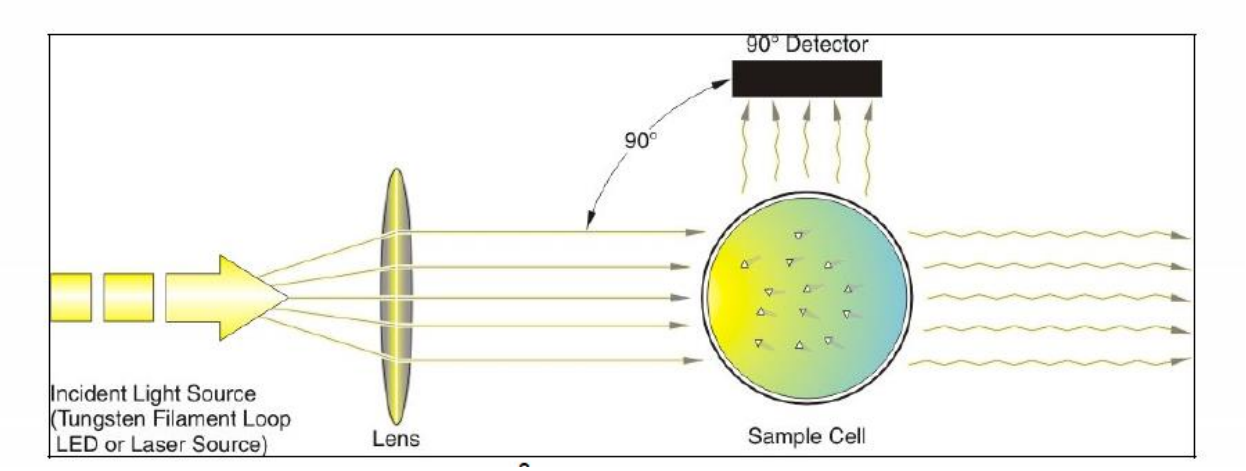


Figure 1. Scattering turbidimeter

3. Materials:

Apparatus:

-Turbidity meter, standards

-Empty vial to hold the samples

-Clean and dry wipe

4. Procedure:

-Calibrate the turbidmeter according to the model you are using (write in detail since each device has a different procedure and different standards.)

-Wipe the vials containing the standards and the samples with a clean cloth so that finger tips or the dirt won't affect the reading of the turbidity meter.

-Place the vials containing the standards in the turbidmeter and wait till its calibrated; you should be able to get the total suspended solids for each standard from the instructor.

-Place the sample vials after cleaning it in the turbidmeter and record the results in your table.

Experiment #3

DETERMINATION OF ACIDITY

1. Objective:

To be familiar with the concepts of acidity, and the measurement of acidity in water.

2. Theory:

Acidity is a measure of the capacity of water to neutralise bases. Acidity is the sum of all titrable acid present in the water sample. Strong mineral acids, weak acids such as carbonic acid, acetic acid present in the water sample contributes to acidity of the water. Usually dissolved carbon dioxide (CO2) is the major acidic component present in the unpolluted surface waters.

The volume of standard alkali required to titrate a specific volume of the sample to pH 8.3 is called phenolphthalein acidity (Total Acidity).

The volume of standard alkali required to titrate a specific volume of the water sample (wastewater and highly polluted water) to pH 3.7 is called methyl orange acidity (Mineral Acidity).

3. Materials:

Apparatus:

-Burettes, 25 mL

-Conical Flask

-Magnetic stirrer and rod

-Beaker

-Pipet

-Measuring cylinder

Solutions

-NaOH 0,02N

-Phenolphtalein indicator

-Methyl orange indicator

4. Procedure (Figure 1)**:**

-Rinse the burette with 0.02N sodium hydroxide and then discard the solution.

-Fill the burette with 0.02N sodium hydroxide and adjust the burette.

-Fix the burette to the stand.

-A sample size is chosen as the titre value does not exceed 20mL of the titrant. For highly concentrated samples, dilute the sample. Usually, take 100 mL of a given sample in a conical flask using pipette.

-Add few drops of methyl orange indicator in the conical flask.

-The colour changes to orange. Now titrate the sample against the 0.02N sodium hydroxide solution until the orange colour faints.

-Note down the volume (S1) consumed for titration. This volume is used for calculating the mineral acidity.

-To the same solution in the conical flask add few drops of phenolphthalein indicator.

-Continue the titration, until the colour changes to faint pink colour.

-Note down the total volume (S2) consumed for titration. This volume is used for calculating the total acidity.

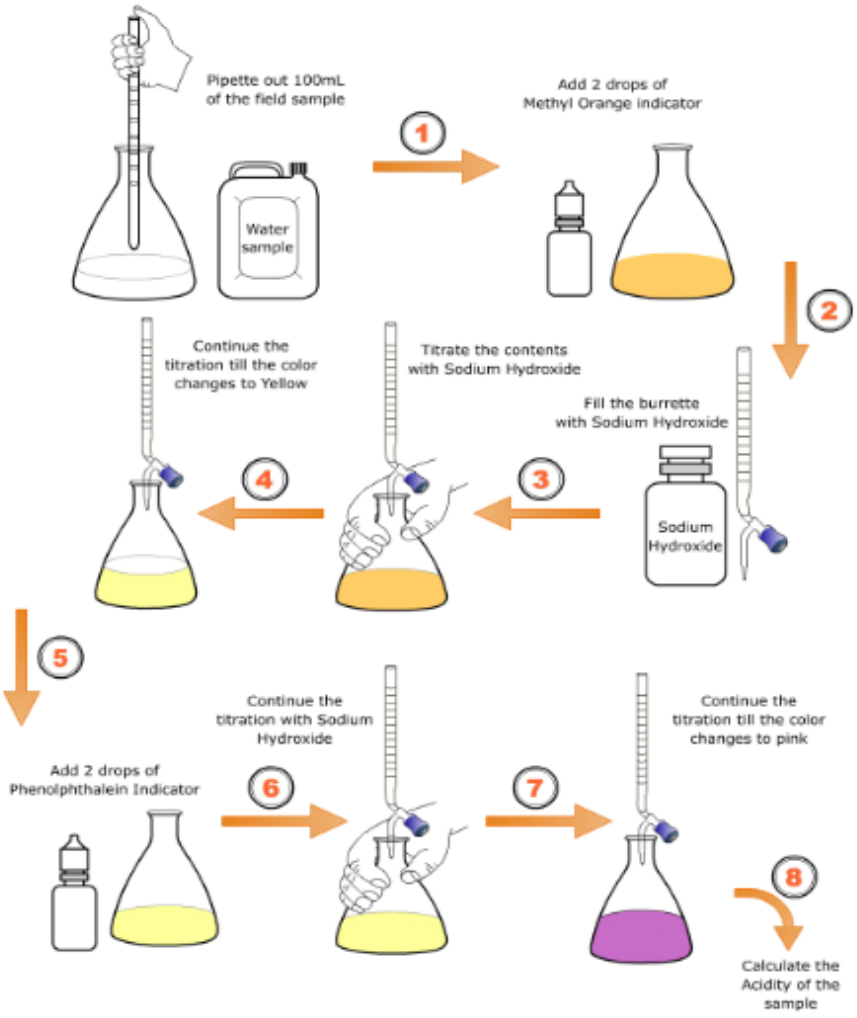


Figure 1. Procedure chart for acidity determination

5. Calculations

Mineral Acidity (mg $CaCO_3$ /L) = $\frac{S1 \times N \times 50 \times 1000}{mL\ Sample}$

S1: mL 0.02 N NaOH used for methyl-orange end point

N: Normality of NaOH , 0.02N

Total Acidity (mg $CaCO_3$ /L) = $\frac{S2 \times N \times 50 \times 1000}{mL\ Sample}$

S2: mL 0.02 N NaOH used for phenolphtalein end point

N: Normality of NaOH , 0.02N

Experiment #4

DETERMINATION OF ALKALINITY

1. Objective:

To be familiar with the concepts of alkalinity and acidity, and the measurement of alkalinity in water.

2. Theory:

Alkalinity is primarily a way of measuring the acid neutralizing capacity of water. In other words, its ability to maintain a relatively constant pH. The possibility to maintain constant pH is due to the hydroxyl, carbonate and bicarbonate ions present in water. The ability of natural water to act as a buffer is controlled in part by the amount of calcium and carbonate ions in solution. Carbonate ion and calcium ion both come from calcium carbonate or limestone. So water that comes in contact with limestone will contain high level of both Ca^{++} and CO_3^{2-} ions and have elevated hardness and alkalinity.

Alkalinity is important for fish and aquatic life because it protects or buffers against rapid pH changes. Higher alkalinity levels in surface waters will buffer acid rain and other acid wastes and prevent pH changes that are harmful to aquatic life. Also, large amount of alkalinity in parts bitter taste in water.

The principal objection of alkaline water is the reactions that can occur between alkalinity and certain cations in waters. The resultant precipitate can corrode pipes and other accessories of water distribution systems.

Alkalinity is the ability of water to resist change in pH, which is due to presence of certain species such as hydroxide ions OH^-, bicarbonate ions HCO_3^- and carbonate ions CO_3^{2-}. The alkalinity of natural waters is due primarily to the salts of week acids. Bicarbonates represent the major form of alkalinity. Alkalinity can be expressed as follows:

Total Alkalinity= $[HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$

Carbonate Alkalinity = $[CO_3^{-2}] + [OH^-] - [H^+] - [H_2CO_3]$

Caustic Alkalinity = $[OH^-] - [H^+] - [HCO^-] - 2[H_2CO_3]$

The alkalinity of water can be determined by titrating the water sample with sulphuric acid of known values of pH, volume and concentrations. Based on stoichiometry of the reaction and number of moles of Sulphuric acid needed to reach the end point, the concentration of

alkalinity in water is calculated. A titration curve of a bicarbonate containing water is presented in Figure 1.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. As alkalinity of many surface waters constitute of carbonates, bicarbonate and hydroxide contents, it is assumed to be an indicator of these constituents as well. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than or only slightly greater than that of the water supply. In wastewater treatment, alkalinity is an important parameter. In determining the amenability of wastes to the treatment process and control of processes such as anaerobic digestion, where bicarbonate alkalinity, total alkalinity, and any fraction contributed by volatile acid salts become considerations.

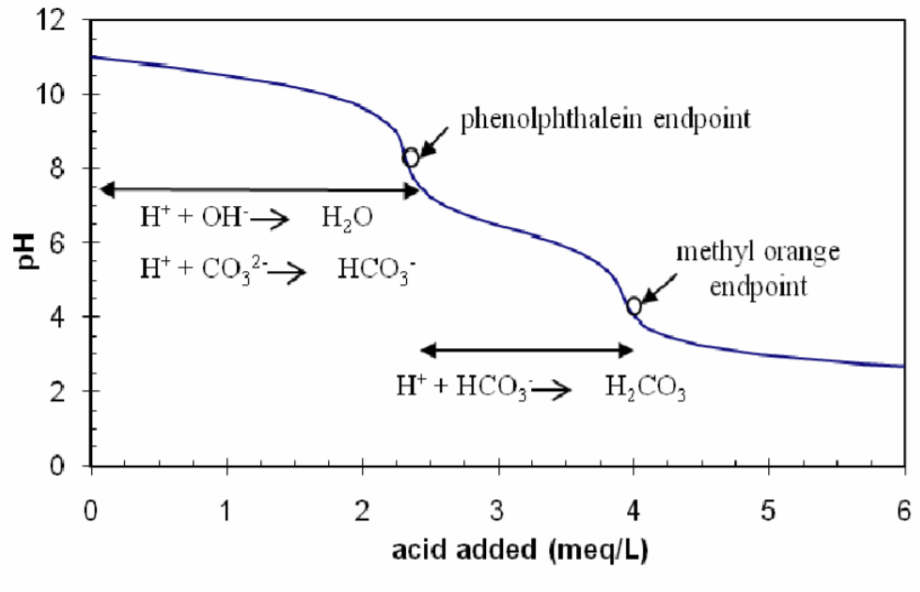


Figure 1. A titration curve of a bicarbonate containing water

3. Materials:

Apparatus:

-Burettes, 25 mL

-Conical Flask

-Magnetic stirrer and rod

-Beaker

-Pipet

-Measuring cylinder

-pH meter

Solutions

-NaOH 0,02N

-H2SO4 0,02N

-Phenolphtalein indicator

-Methyl orange indicator

4. Procedure (Figure 2)**:**

-Pipet exactly 100 mL of sample into conical flask and drop in a magnetic rod.

-Mount a 25 ml burette and fill it to the mark with 0.02 N sulfuric acid solution.

-Add 5 drops of phenolphthalein indicator to the sample, if the solution turns pink, add acid's slowly till pink color disappears. Record the volume as S.

-Add 5 drops of methyl orange indicator to the same sampling at the end of first titration and add 0.02 N sulfuric acid slowly till orange color turns to pink.

5. Calculations

$$\text{Alkalinity (mg } CaCO_3\text{ /L)} = \frac{S \times N \times 50000}{\text{mL Sample}}$$

S: mL 0.02 N H_2SO_4 used for methyl-orange end point

N: Normality of H_2SO_4 , 0.02N

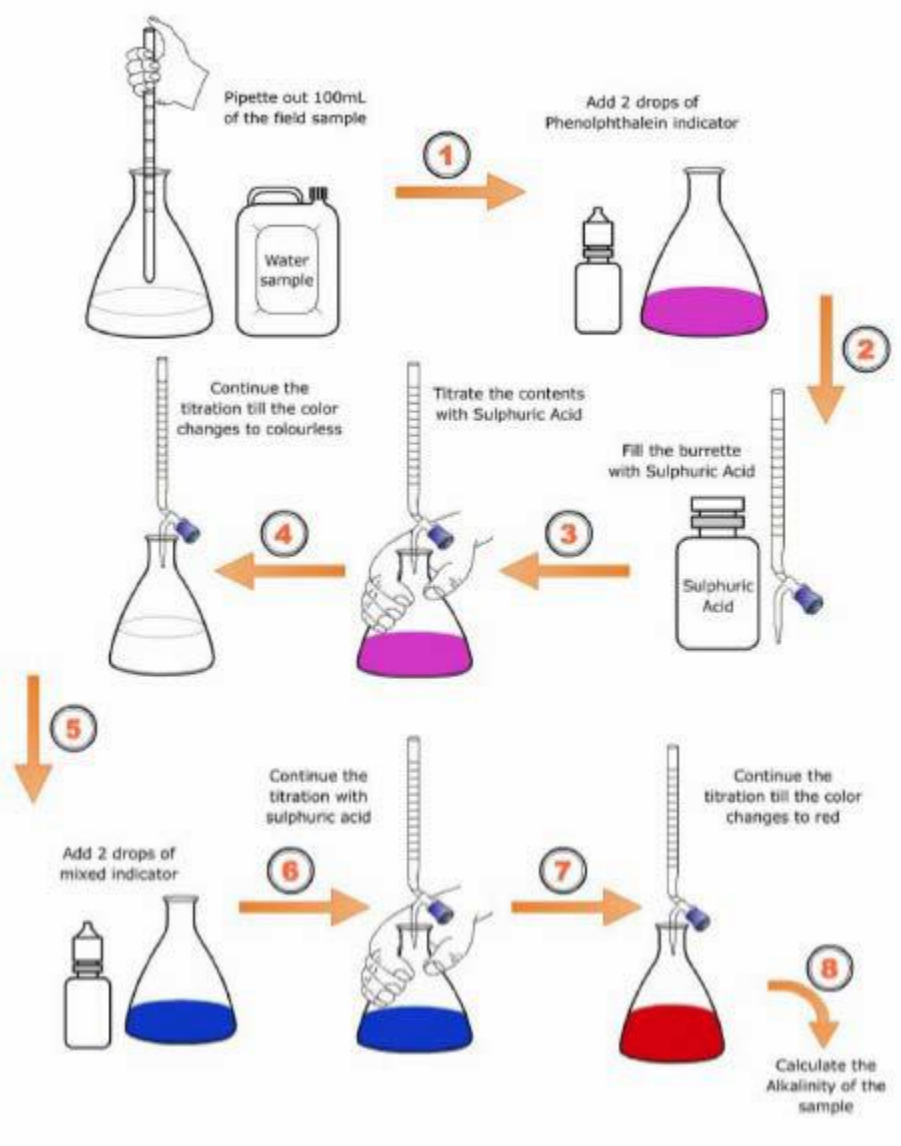


Figure 2. Procedure chart for alkalinity determination

Experiment #5

DETERMINATION OF HARDNESS

1. Objective:

To determine hardness of water and to familiarize the students with complex formation.

2. Theory:

Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present. Other polyvalent cations also may precipitate soap, but they often are in complex forms, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. In conformity with current practice, total hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter.

EDTA Titrimetric Method

Ethylenediaminetetraacetic acid and its sodium salts (abbreviated EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0 ± 0.1, the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of the magnesium and calcium has been complexed the solution turns from wine red to blue, marking the endpoint of the titration.

3. Materials:

Apparatus:

-Burette

-Pipette

-Volumetric flask

-Erlenmeyer flasks

Reagents

-Buffer solution:

Dissolve 16.9 g ammonium chloride (NH4Cl) in 143 mL conc ammonium hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA (available commercially) and dilute to 250

mL with distilled water. Store Solution in a plastic or borosilicate glass container for no longer than 1 month. Stopper tightly to prevent loss of ammonia (NH3) or pickup of carbon dioxide (CO2). Dispense buffer solution by means of a bulb-operated pipet. Discard buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ±0.1 at the titration endpoint.

- Indicators:

Many types of indicator solutions have been advocated and may be used if the analyst demonstrates that they yield accurate values. The prime difficulty with indicator solutions is deterioration with aging, giving indistinct endpoints. For example, alkaline solutions of Eriochrome Black T are sensitive to oxidants and aqueous or alcoholic solutions are unstable. In general, use the least amount of indicator providing a sharp endpoint. It is the analyst's responsibility to determine individually the optimal indicator concentration.

-Eriochrome Black T:

Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid; No. 203 in the Color Index. Dissolve 0.5 g dye in 100 g 2,2,2-nitrilotriethanol (also calledtriethanolamine)or2-methoxymethanol(alsocalledethylene glycol monomethyl ether). Add 2 drops per 50 mL solution to be titrated. Adjust volume if necessary.

Indicators can be used in dry powder form if care is taken to avoid excess indicator. Prepared dry mixtures of these indicators and an inert salt are available commercially. If the endpoint color change of these indicators is not clear and sharp, it usually means that an appropriate complexing agent is required. If NaCN inhibitor does not sharpen the endpoint, the indicator probably is at fault.

-Murexide indicator:

Mix 200 mg Murexide with 100 g NACI and grind the mixture to get homogeneous powder.

-Standard EDTA titrant, 0.01M:

Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate, also called (ethylenedinitrilo)tetraacetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1000 mL.

Because the titrant extracts hardness-producing cations from soft glass containers, store in polyethylene (preferable) or borosilicate glass bottles. Compensate for gradual deterioration by periodic restandardization and by using a suitable correction factor.

-Standard calcium solution: Weigh 1.000 g anhydrous $CaCO_3$ powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL Erlenmeyer flask.

Place a funnel in the flask neck and add, a little at a time, 1+1 HCl until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3N NH4OH or 1+1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water; 1 mL=1.00 mg CaCO3.

-Sodium hydroxide (NaOH), 4N.

4. Procedure:

Total hardness:

-Put 50 ml of the sample into an erlenmeyer flask.

-Add 1-2 ml (10 drops) buffer (NH_4CI-NH_4OH)

-The pH of the solution should be 10±0.1 at this point.

-Add some EBT indicator until you observe the deep purple color.

-Titrate with EDTA until a blue color is reached.

Calcium Hardness:

-Put 50 ml of the smple into an erlenmeyer flask.

- Add 4 drops 4 N NaOH solution.

- The pH of the solution should be 12±0.1 at this point.

- Add some Murexide indicator until you observe the pink color.

- Titrate with EDTA until a purple color is reached.

5. Calculations

1 ml EDTA solution=1 mg hardness as $CaCO_3$

Hardness (EDTA) as mg $CaCO_3$/L=A xB x1000/ mL sample

where:

A:mL titration for sample, and B: mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant.

Experiment #6

DETERMINATION OF CHLORIDE (**Argentometric Method)**

1. Objective:

To measure chloride in water samples.

2. Theory:

Chloride, in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water and wastewater. The salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg Cl^-/l may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1000 mg/L when the predominant cations are calcium and magnesium. The chloride concentration is higher in wastewater than in raw water because sodium chloride (NaCl) is a common article of diet and passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewerage system. It also may be increased by industrial processes. A high chloride content may harm metallic pipes and structures, as well as growing plants.

Sampling and Storage: Collect representative samples in clean, chemically resistant glass or plastic bottles. The maximum sample portion required is 100 mL. No special preservative is necessary if the sample is to be stored.

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

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

3. Materials:

Apparatus:

-Erlenmeyer flask, 250-mL.

-Buret, 50-mL

Solutions:

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

-Standard silver nitrate titrant, 0.0141M (0.0141N): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL. Store in a brown bottle.

-Standard sodium chloride, 0.0141M (0.0141N): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL=500 µg Cl^-

4. Procedure:

Sample preparation:

-Use a 100-mL sample or a suitable portion diluted to 100 mL.

-If the sample is highly colored, add 3 mL $Al(OH)_3$ suspension, mix, let settle, and filter.

-If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

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 nonchloride-type reference electrode.

-Add 1.0 mL K_2CrO_4 indicator solution.

-Titrate with standard $AgNO_3$ titrant to a pinkish yellow endpoint. Be consistent in endpoint recognition.

-Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method out lined above.

-A blank of 0.2 to 0.3 mL is usual.

5. Calculations

mg Cl^-/L= (A-B)xN x35450/ mL sample

where:

A:mL titration for sample,

B:mL titration for blank, and

N: normality of AgNO3.

mg NaCl/L=(mg Cl^-/L)x1.65

Experiment #7

DETERMINATION OF DISSOLVED OXYGEN

1. Objective:

To correctly perform dissolved oxygen measurements

2. Theory:

All gases of the atmosphere are soluble in water to some degree. Oxygen is classified as poorly soluble, and its solubility is affected both by atmospheric pressure, and physical and chemical properties of water such as temperature, salinity, pollutants, etc. The solubility of atmospheric oxygen in fresh waters ranges from 14.6 mg/L at 0^oC to about 7 mg/L at 35^oC under 1 atm. of pressure. Most of the critical conditions related to dissolved-oxygen deficiency, both in natural waters and biological wastewater treatment, occur during the warmer months when temperatures are high and solubility of oxygen is at a minimum. The low solubility of oxygen is a major factor limiting the purification capacity of natural waters. In aerobic biological treatment processes, the limited solubility of oxygen is also of great importance, because it governs the rate at which oxygen will be absorbed by the medium and therefore the cost of aeration. Hence, DO analysis is a key test both in natural waters and water pollution control practice.

The Winkler test is used to determine the concentration of dissolved oxygen in water samples. The Winkler Method uses titration to determine dissolved oxygen in the water sample. A sample bottle is filled completely with water (no air is left to skew the results). The dissolved oxygen in the sample is then "fixed" by adding a series of reagents that form an acid compound that is then titrated with a neutralizing compound that results in a color change. The point of color change is called the "endpoint," which coincides with the dissolved oxygen concentration in the sample.

Reactions;

If the sample doesn't contain oxygen, when $MnSO_4$ and alkali iodide (NaOH + KI) was added, the white $Mn(OH)_2$ flocs will ocur.

$Mn^{+2} + 2OH \rightarrow Mn(OH)_2 \downarrow$ (*White precipitate*)

If the sample contain oxygen, when $MnSO_4$ and alkali iodide (NaOH + KI) was added, the brown MnO_2 flocs will ocur.

$Mn^{+2} + 2OH + 1/2O_2 \rightarrow MnO)_2 \downarrow + H_2O$ (*brown precipitate*)

3. Materials:

Apparatus:

-Burettes, 25 mL

-Incubation bottles

-Erlenmayer

-Pipette

-Measuring cylinder

Solutions :

-Manganese sulfate solution:

Dissolve 480 g $MnSO_4$.4H2O, 400 g $MnSO_4$.2H_2O or 364 g $MnSO_4$.H_2O in distilled water, filter, and dilute to 1L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

- Alkali-iodide-azide reagent:

Dissolve 10 g sodium azid (NaN_3) in 500 mL distilled water, add 480 g Sodium hyroxide (NaOH) and 750 g Sodium iodide (NaI) into this solution. Stir until dissolved.

- Sulfuric acid:

One mL is equivalent to ~ 3mL alkali-iodide-azide reagent.

- Starch solution:

Dissolve 2 g laboratory-grade soluble starch and 0.2 g salicyclic acid as preservative in 100 mL hot distilled water.

- Standard sodium thiosulfate titrant: Dissolve 6.205 g $Na_2S_2O_3$ .5H_2O in distiller water and add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

- Standard potassium bi-iodate solution (0.0021M):

Dissolve 812.4 mg $KH(IO_3)$ in distilled water and dilute to 1000 mL.

- Standardization:

Dissolve e ~ 2 g KI, free from iodate in an Erlenmeyer flask with 100 to 150 mL distilled water; add 1 mL 6N H_2SO_4 or a few drops of conc. H_2SO_4 and 20 mL standard bi-iodate solution. Dilute to 200 mL and titrate librated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solution is of equal, 20 mL 0.025M Na_2S_2O3 should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025M.

4. Procedure (Figure 1)**:**

-Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) bottle brim-full with sample water.

-Add 2 mL of manganese sulfate to the bottle by inserting the pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.

-Add 2 mL of alkali-iodide-azide reagent in the same manner.

-Check the bottles for air bubbles; there musn't be air bubbles. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.

-Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully mix the sample several times to dissolve the floc.

-Take 203 ml of the sample to a erlanmayer.

-Start titration the sample with sodium thiosulfate to a light yellow color.

-Add 2 drops of starch solution so a blue color forms.

-Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color.

-The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in equals 1 mg/L dissolved oxygen.

5. Calculations

1 ml 0,025N $Na_2S_2O_3$(sodium thiosulfate) = 1 mg/L Dissolved Oxygen

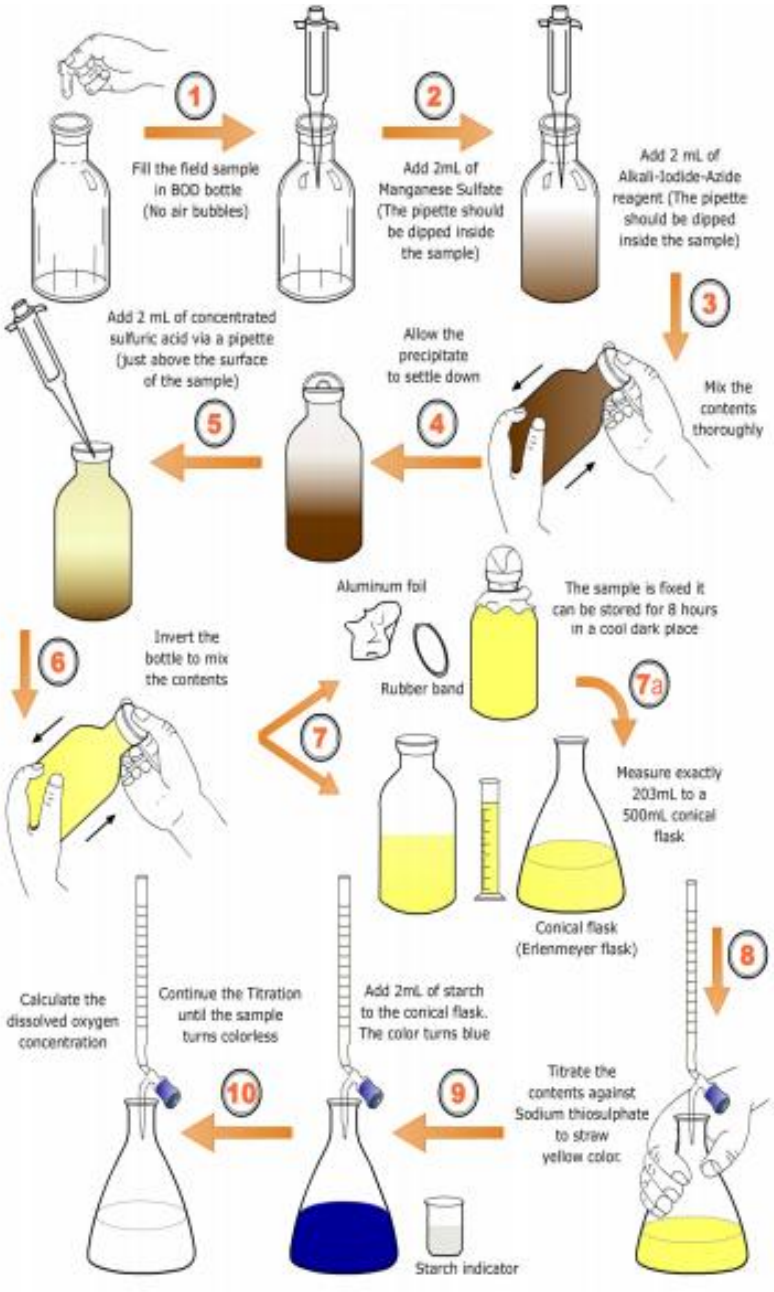


Figure 1. Procedure chart for dissolved oxygen determination

Experiment #8

DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND

1. Objective:

To correctly perform BOD measurements

2. Theory:

Biochemical oxygen demand (BOD) is defined as the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions. The BOD test is widely used to determine the pollutional strength of domestic and industrial wastewaters in terms of the oxygen that they will require if discharged into natural watercourses in which aerobic conditions exist. The test is one of the most important both in regulatory work and in studies designed to evaluate the purification capacity of receiving water bodies. Its disadvantage is the long time required by the test, generally taking 5 days.

In the presence of free oxygen, aerobic bacteria use the organic matter found in wastewater as "food". The BOD test is an estimate of the "food" available in the sample. The more "food" present in the wastewater, the more Dissolved Oxygen (DO) will be required. The BOD test measures the strength of the wastewater by measuring the amount of oxygen used by the bacteria as they stabilize the organic matter under controlled conditions of time and temperature.

The DO content is determined and recorded and the bottle is incubated in the dark for five days at 20°C. At the end of five days, the final DO content is determined and the difference between the final DO reading and the initial DO reading is calculated. The decrease in DO is corrected for sample dilution, and represents the biochemical oxygen demand of the sample

3. Materials:

Apparatus:

-Burettes, 25 mL

-Incubation bottles

-Erlenmayer

-Pipette

-Measuring cylinder

Solutions:

-Manganese sulfate solution:

Dissolve 480 g $MnSO_4$.4H2O, 400 g $MnSO_4$.2H_2O or 364 g $MnSO_4$.H_2O in distilled water, filter, and dilute to 1L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

- Alkali-iodide-azide reagent:

Dissolve 10 g sodium azid (NaN_3) in 500 mL distilled water, add 480 g Sodium hyroxide (NaOH) and 750 g Sodium iodide (NaI) into this solution. Stir until dissolved.

- Sulfuric acid:

One mL is equivalent to ~ 3mL alkali-iodide-azide reagent.

- Starch solution:

Dissolve 2 g laboratory-grade soluble starch and 0.2 g salicyclic acid as preservative in 100 mL hot distilled water.

- Standard sodium thiosulfate titrant:

Dissolve 6.205 g $Na_2S_2O_3$ .5H_2O in distiller water and add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

- Standard potassium bi-iodate solution (0.0021M):

Dissolve 812.4 mg $KH(IO_3)$ in distilled water and dilute to 1000 mL.

- Standardization:

Dissolve e ~ 2 g KI, free from iodate in an Erlenmeyer flask with 100 to 150 mL distilled water; add 1 mL 6N H_2SO_4 or a few drops of conc. H_2SO_4 and 20 mL standard bi-iodate solution. Dilute to 200 mL and titrate librated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solution is of equal, 20 mL 0.025M Na_2S_2O3 should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025M.

4. Procedure(Figure 1)**:**

-Chose the dilution rate from table according to approximately BOD value of the sample. For example; approximately BOD value of the sample is 7500 mg/L. It shows the 0,05. From the sample for 1L, (0,05/100)x1000=0,5 ml must be taken. For dilution, under and above of 0,05 from the table must be prepared for correction.

-Take the sample to measuring cylinder which found in the previous step.

-Add 1 ml seed and fill the measuring cylinder to 1L with dilution water

-Fill the incubation bottles brim-full with this sample.

-Check the bottles for air bubbles, there musn't be air bubbles.

-Close the stopper of bottles and add water to prevent the oxygen transfer to sample from air.

-Take 3 of that samples, and measure the dissolved oxygen concentration by winkler method in maximum 30 minutes immediately to know initial dissolved oxygen concentration.

After 5-days

-Take the bottles from incubator.

-Add 2 mL of manganese sulfate to the bottle by inserting the pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.

-Add 2 mL of alkali-iodide-azide reagent in the same manner.

-Check the bottles for air bubbles; there musn't be air bubbles. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.

-Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully mix the sample several times to dissolve the floc.

-Take 203 ml of the sample to a erlanmayer.

-Start titration the sample with sodium thiosulfate to a light yellow color.

-Add 2 drops of starch solution so a blue color forms.

-Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color..

-The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 7 and 9 equals 1 mg/L dissolved oxygen.

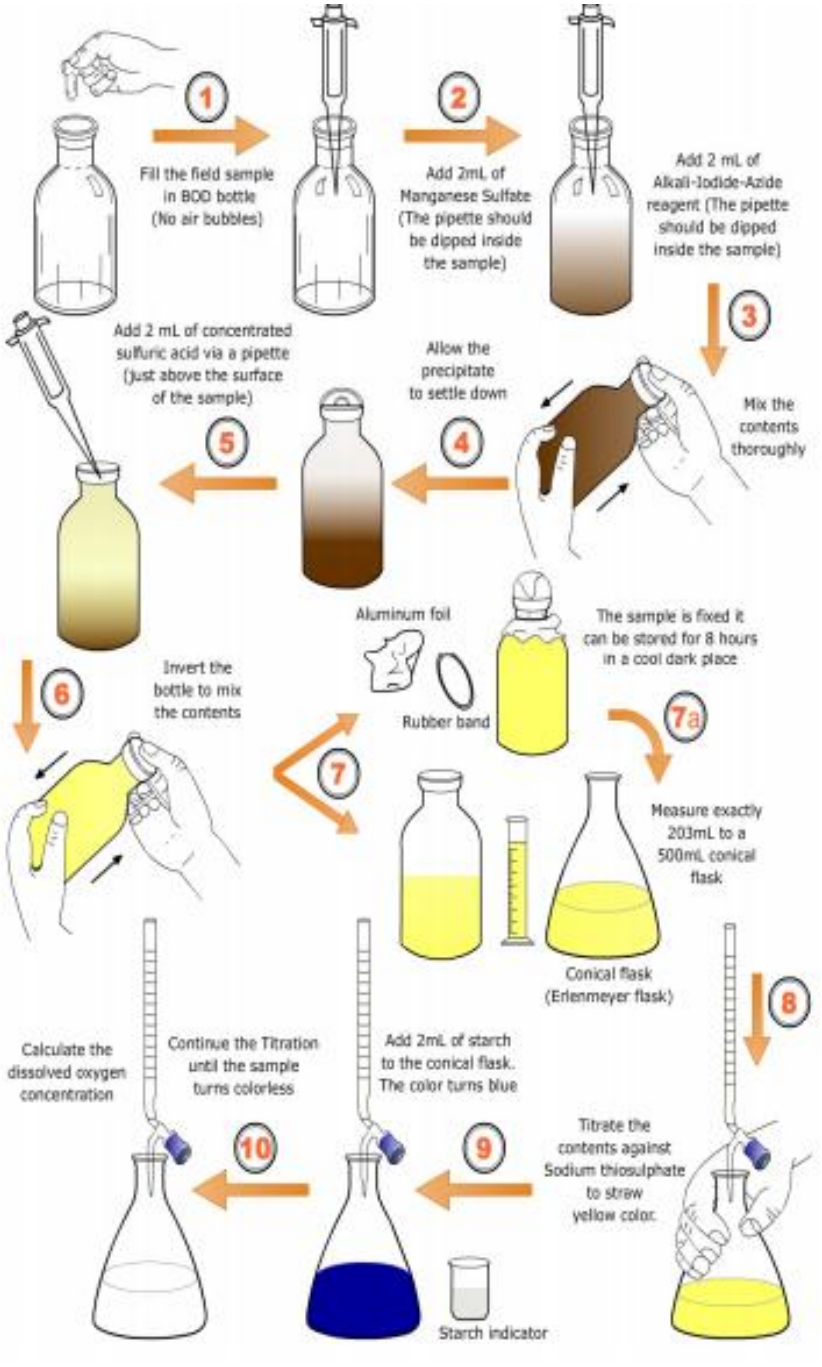


Figure 1. Procedure chart for BOD determination

5. Calculations

Environmental sample							
Bottle number	**Sample size (mL)**	**Initial DO reading (D_1)**	**Date/ time of reading**	**Final DO reading (D_2)**	**Date /time of reading**	**BOD $\frac{D_1 - D_2}{P}$**	**BOD average (mg/L)**

If dilution-water demand is <0.2 milligrams per liter (mg/L), use

$$BOD_5\ (mg/L) = \frac{D_1 - D_2}{P}$$

where

D_1 = initial sample dissolved-oxygen (DO) concentration (in mg/L)
D_2 = sample DO (in mg/L) after 5 days
P = decimal volumetric fraction of sample used

Experiment #9

DETERMINATION OF CHEMICAL OXYGEN DEMAND

1. Objective:

To measure Chemical Oxygen Demand.

2. Theory:

The chemical oxygen demand (COD) determines the amount of oxygen required for chemical oxidation of organic matter using a strong chemical oxidant, such as, potassium dichromate under reflux conditions. This test is widely used to determine:

a) Degree of pollution in water bodies and their self-purification capacity,

b) Efficiency of treatment plants,

c) Pollution loads, and

d) Provides rough idea of Biochemical oxygen demand (BOD) which can be used to determine sample volume for BOD estimation.

The limitation of the test lies in its inability to differentiate between the biologically oxidizable and biologically inert material and to find out the system rate constant of aerobic biological stabilization.

Most of the organic matters are-destroyed when boiled with a mixture of potassium dichromate and sulphuric acid producing carbon dioxide and water. A sample is refluxed with a known amount of potassium dichromate in sulphuric acid medium and the excess of dichromate is titrated against ferrous ammonium sulphate. The amount of dichromate consumed is proportional to the oxygen required to oxidize the oxidizable organic matter.

There are two methods available for COD determination namely open reflux and closed reflux.

Open Reflux Principle:

Suitable for a wide range of wastes with a large sample size.

Due to it higher oxidizing ability dichromate reflux method is preferred over other procedures using other oxidants (e.g. potassium permanganate).

Oxidation of most organic compounds is up to 95-100% of the theoretical value.

Closed Reflux Principle:

This method is conducted with ampules and culture tubes with pre-measured reagents.

Measurement of sample volume and reagent volume are critical.

This method is economical in the use of metallic salt reagents and generate smaller quantity of hazardous wastes.

Volatile organic compounds (VOC) gets completely oxidized in a closed system than the open because of longer contact time with oxidants.

Chemical Reactions:

$C_nH_aO_bN_c + d\,Cr_2O_7^{2-} + (8d+c)\,H^+ \Rightarrow nCO_2 + [(a+8d-3c)/2]H_2O + c\,NH_4^+ + 2dCr^{3+}$ (1)

Here d= (2n/3) + (a/6)-(b/3)-(c/2)

During experiment, excess dichromate concentration is determined by titrating it with ferrous ammonium sulfate (FAS). The reaction is given by:

$6Fe_2^+ + Cr_2O_7^{2-} + 14\,H^+ \Rightarrow 6Fe^{3+} + 2Cr^{3+} + 7H_2O$ (2)

Here d= (2n/3) + (a/6)-(b/3)-(c/2)

3. Materials:

Apparatus:

-Burettes, 25 mL

-Conical Flask

-Magnetic stirrer and rod

-Beaker

-Pipet

-Measuring cylinder

-tubes with caps

-distilled water

-block digester

Solutions

-Standard Potassium dichromate ($K_2Cr_2O_7$) digestion solution, 0.01667M:

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 conc. H_2SO4, and 33.3 g HgSO4. Dissolve, cool to room temperature, and dilute to 1000 mL.

-Sulfuric acid reagent:

Add H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4 or 10.12 g silver sulphate/L H_2SO_4. Let stand 1 to 2 d to dissolve and mix. This accelerates the oxidation of straightchain aliphatic and aromatic compounds.

(1 Kg = 543.47826 mL of H_2SO_4 and take 20.24 g of Ag_2SO_4 to 2 L of H_2SO_4 or 22.264 g of Ag_2SO_4 to 2.2 L of H_2SO_4)

-Ferroin Indicator solution:

This indicator is used to indicate change in oxidation-reduction potential of the solution and indicates the condition when all dichromate has been reduced by ferrous ion. It gives a very sharp brown color change which can be seen in spite of blue color generated by the Cr^{3+} ions formed on reduction of the dichromate.

-Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10M:

Dissolve 39.2 g Fe $(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 mL conc. H_2SO_4, cool, and dilute to 1000 mL. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows: Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted Ferroin indicator and titrate with FAS titrant.

Molarity of FAS solution = [V $K_2Cr_2O_7$ ×0.1] / (VFAS)

Where: V$K_2Cr_2O_7$ = volume of $K_2Cr_2O_7$ (mL); VFAS = volume of FAS (mL)

4. Procedure (Figure 1)**:**

-Wash culture tubes and caps with 20% H2SO4 before using to prevent contamination.

-Place sample (2.5 mL) in culture tube and add K2Cr2O7 digestion solution (1.5 mL).

-Carefully run sulphuric acid reagent (3.5 mL) down inside of vessel so an acid layer is formed under the sample-digestion solution layer and tightly cap tubes or seal ampules, and invert each several times to mix completely.

-Place tubes in block digester preheated to 150°C and reflux for 2 h behind a protective shield.

-Cool to room temperature and place vessels in test tube rack. Some mercuric sulfate may precipitate out but this will not affect the analysis.

-Add 1 to 2 drops of Ferroin indicator and stir rapidly on 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.

-In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

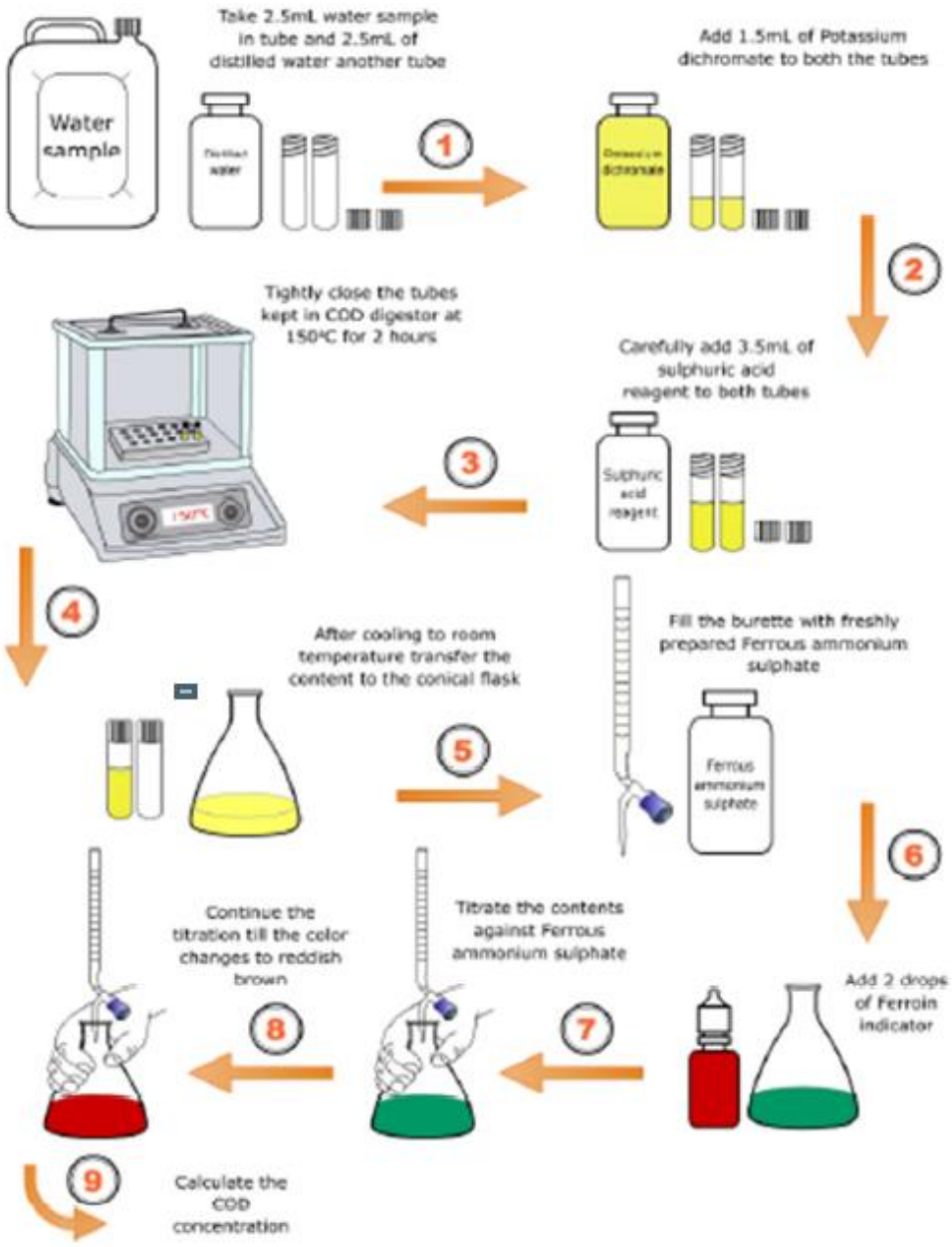


Figure 1. Procedure chart for COD determination

5. Calculations

COD (mg O2 /L) = [(A-B) × M ×8000) / (V sample)

Where: A = volume of FAS used for blank (mL)

B = volume of FAS used for sample (mL)

M = molarity of FAS

8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

Experiment #10

DETERMINATION OF SOLID MATTERS

1. Objective:

The objective of this experiment is to determine the various types of solids in tap water, drinking water, and secondary effluent

2. Theory:

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely in a number of ways. Waters with high dissolved solids generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer. For these reasons, a limit of 500 mg dissolved solids per liter is desirable for drinking waters. Highly mineralized waters also are unsuitable for many industrial applications. Waters high in suspended solids may be esthetically unsatisfactory for such purposes as bathing. Solids analysis are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

Total Solids is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature (103-1050C). Total suspended solids refer to the nonfilterable residue retained by a standard filter disk and dried at 103-1050C. Total dissolved solids refer to the filterable residue that pass through a standard filter disk and remain after evaporation and drying to constant weight at 103-1050C.

The environmental impacts of solids are that solids in all forms have detrimental effects on quality since they cause putrifaction problems. “Suspended solids” is the portion retained on the filter. Suspended solids exclude light, thus reducing the growth of oxygen producing plants. Solids impair aesthetic acceptability of water. The type of filter holder, the pore size, porosity, area, and thickness of the filter and the physical nature, particle size, and amountof material deposited on the filter are the principal factors affecting separation of suspended from dissolved solids. “Dissolved solids” is the portion of solids that passes through a filter of 2.0 m (or smaller) nominal pore size under specified conditions. Fixed solid is the term applied to the residue of total, suspended, or dissolved solids after heating to dryness for a specified time at a specified temperature. The weight loss on ignition is called “volatile solids.” Determinations of fixed and volatile solids do not distinguish precisely between

inorganic and organic matter because the loss on ignition is not confined to organic matter. It includes losses due to decomposition or volatilization of some mineral salts. Better characterization of organic matter can be made by such tests as total organic carbon. Settleable solids is the term applied to the material settling out of suspension within a defined period. Settleable solids in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (mL/L) or a weight (mg/L) basis. It may include floating material, depending on the technique .

3. Materials:

Apparatus:

-drying oven
-muffle furnace.
-desiccator
-crucible
-analytical balance
-filtration apparatus
-pipettes
-measuring cylinders
-imhof settling cones
-membrane filter (0.45 μm diameter pore size)
-forceps

4. Procedure:

Total Solids (Figure 1)

-Ignite a clean porcelain crucible at 550^0C in a muffle furnace for 1 hr.
-Cool the dish, weigh and keep it in a desiccator.
-Transfer carefully 20 ml of sample into the crucible
-Place the evaporated sample in an oven adjusted at 103^0C and dry it for 1 hr
-Repeat drying at 103^0C till constant weight is obtained.

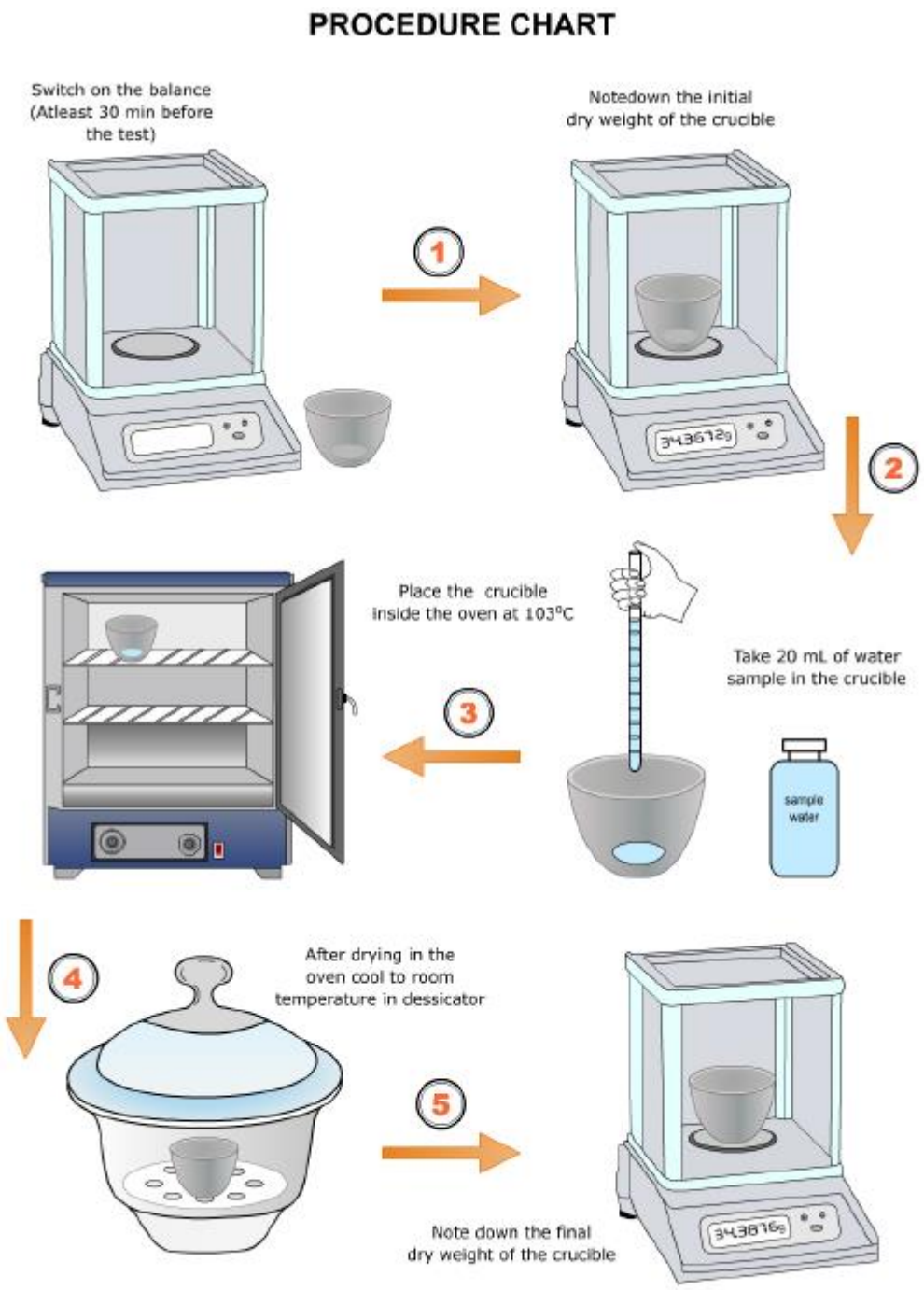


Figure 1. Procedure chart for total solids determination

Total suspended solids (Figure 2):

-Place a membrane filter (0.45 µm pore size) to an oven and dry it for 1 hr at 103^0C.

-After drying, the membrane filter is kept in a desiccator.

-Weigh the membrane filter and place it on a filtration apparatus.

-Pour 20 ml of sample. Wash pipette with distilled water.

-After filtration, dry the membrane filter at 103^0C for 1 hr

-Weigh till constant weight is obtained.

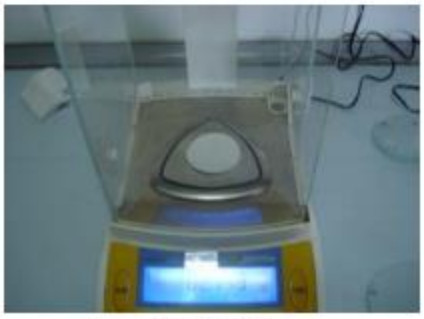
Weigh 0,45 µm membran filter

Place filter on the membrane filter apparatus

Membrane filter apparatus

Dry in oven

Cool in desiccator



Dry weight of the filter

Figure 2. Procedure chart for total suspended solids determination

Total Dissolved Solids

Total Dissolved Solids can be calculated from following equation:

mg/l total dissolved solids = total solids – total suspended solids

-If only total dissolved solids are to be measured, heat the clean porcelain crucible 103-105 ºC for one hour.
-Cool, desiccate, weigh and store in desiccator.
-Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 20 mL to the funnel by means of a 20 mL graduated cylinder.
-Filter the sample through membrane filter
-Transfer 20 mL of the filtrate to a weighed porcelain crucible. Dry the sample for at least one hour at 103-105ºC.
-Cool in a desiccator and weigh until a constant weight is obtained.

Total Volatile and Fixed Solids (Figure 3)
Material that can be volatilized and burned off when ignited at 550 °C is classified as volatile. Fixed solids (FS) comprise the residue that remains after a sample has been ignited.

-Ignite residues produced from Total Solids determination to constant weight in a muffle furnace at 550±50ºC (Usually 15-20 min. ignition is required).
-Allow the porcelain crucible to cool partially in air until most of the heat has been dissipated and transfer to a desiccator for final cooling in a dry atmosphere.
-Weight the dish as soon as it has cooled completely.
-Repeat weighing until a constant weight is obtained

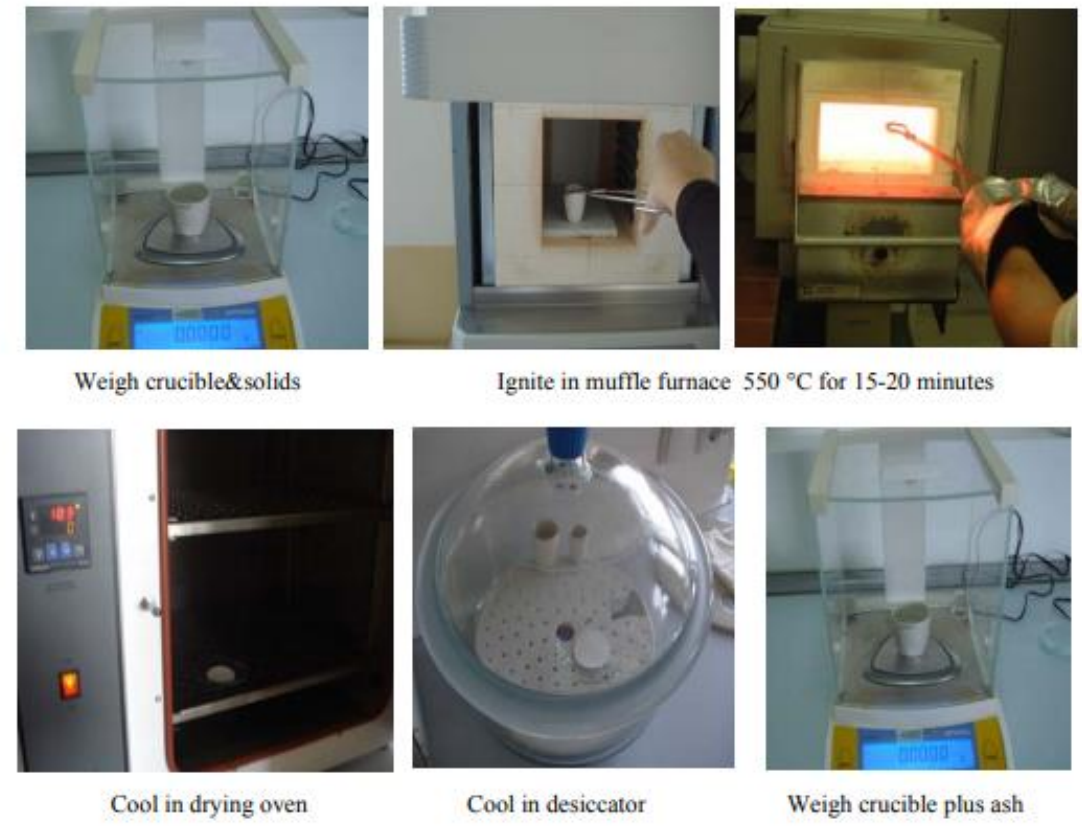


Figure 3. Procedure chart for Total Volatile and Fixed Solids determination

Settleable Solids (Figure 4)

-Fill an Imhoff cone to the 1-L mark with a well-mixed sample.

-Settle for 45 min,

-Gently agitate sample near the sides of the cone with a rod or by spinning

-Settle 15 min longer, and record volume of settleable solids in the cone as milliliters per liter.

-If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids.

Figure 4. Procedure chart for settleable solids determination

5. Calculations

Total Solids

$$\text{TS (mg/L)} = \frac{(\text{Residue} + \text{porcelain crucible})(\text{mg}) - (\text{porcelain crucible})(\text{mg})}{\text{sample volume (mL)}} \text{x} 1000 \left(\frac{\text{mL}}{\text{L}}\right)$$

Total suspended solids

$$\text{TSS (mg/L)} = \frac{(\text{Residue} + \text{filter})(\text{mg}) - (\text{filter})(\text{mg})}{\text{sample volume (mL)}} \text{x} 1000 \left(\frac{\text{mL}}{\text{L}}\right)$$

Total Dissolved Solids:

1) TDS = total solids – total suspended solids

2) $$\text{TDS (mg/L)} = \frac{(\text{Filtered residue} + \text{porcelain crucible})(\text{mg}) - (\text{porcelain crucible})(\text{mg})}{\text{sample volume (mL)}} \text{x} 1000 \left(\frac{\text{mL}}{\text{L}}\right)$$

Total Volatil and Fixed Solids

Total Volatile Solids

$$\text{TVS (mg/L)} = \frac{(\text{Residue at } 105^{\circ}\text{C} + \text{porcelain crucible})(\text{mg}) - (\text{residue at } 550^{\circ}\text{C} + \text{porcelain crucible})(\text{mg})}{\text{sample volume (mL)}} \text{x} 1000 \left(\frac{\text{mL}}{\text{L}}\right)$$

Total Fixed Solids

$$\text{TFS (mg/L)} = \frac{(\text{Residue at } 105^{\circ}\text{C} + \text{porcelain crucible})(\text{mg}) - (\text{porcelain crucible})(\text{mg})}{\text{sample volume (mL)}} \text{x} 1000 \left(\frac{\text{mL}}{\text{L}}\right)$$

Settleable Solids

The practical lower limit of measurement depends on sample composition and generally is in the range of 0.1 to 1.0 mL/L.

Settling Test Data (in an Imhoff Cone for 1 hour)

Sample Volume = 1 L

t (min)	10	20	30	40	50	60
mL/L						

Experiment #11

DETERMINATION OF IRON (Phenanthroline Method)

1. Objective:

To determine iron in water sample.

2. Theory:

Iron (Fe) is the first element in Group VIII of the periodic table; it has an atomic number of 26, an atomic weight of 55.85, and common valences of 2 and 3 (and occasionally valences of 1, 4, and 6). The average abundance of Fe in the earth's crust is 6.22%; in soils Fe ranges from 0.5 to 4.3%; in streams it averages about 0.7 mg/L; and in groundwater it is 0.1 to 10 mg/L. Iron occurs in the minerals hematite, magnetite, taconite, and pyrite. It is widely used in steel and in other alloys. The solubility of ferrous ion (Fe^{2+}) is controlled by the carbonate concentration. Because groundwater is often anoxic, any soluble iron in ground water is usually in the ferrous state. On exposure to air or addition of oxidants, ferrous iron is oxidized to the ferric state (Fe^{3+}) and may hydrolyze to form red, insoluble hydrated ferric oxide. In the absence of complex-forming ions, ferric iron is not significantly soluble unless the pH is very low. Elevated iron levels in water can cause stains in plumbing, laundry, and cooking utensils, and can impart objectionable tastes and colors to foods. The United Nations Food and Agriculture Organization recommended level for irrigation waters is 5 mg/L. The U.S. EPA secondary drinking water standard MCL is 0.3 mg/L.

Sampling and Storage

Plan in advance the methods of collecting, storing, and pretreating samples. Clean sample container with acid and rinse with reagent water. Equipment for membrane filtration of samples in the field may be required to determine iron in solution (dissolved iron). Dissolved iron, considered to be that passing through a 0.45µm membrane filter, may include colloidal iron. The value of the determination depends greatly on the care taken to obtain a representative sample. Iron in well or tap water samples may vary in concentration and form with duration and degree of flushing before and during sampling. When taking a sample portion for determining iron in suspension, shake the sample bottle often and vigorously to obtain a uniform suspension of precipitated iron.Use particular care when colloidal iron adheres to the sample bottle. This problem can be acute with plastic bottles. For a precise determination of total iron, use a separate container for sample collection. Treat with acid at

the time of collection to place the iron in solution and prevent adsorption or deposition on the walls of the sample container. Take account of the added acid in measuring portions for analysis. The addition of acid to the sample may eliminate the need for adding acid before digestion.

Principle: 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 to 3.3. Three molecules of phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The colored solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 ensures rapid color development in the presence of an excess of phenanthroline. Color standards are stable for at least 6 months.

Interference: Among the interfering substances are strong oxidizing agents, cyanide, nitrite, and phosphates (polyphosphates more so than orthophosphate), chromium, zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5 mg/L, and nickel in excess of 2 mg/L. Bismuth, cadmium, mercury, molybdate, and silver precipitate phenanthroline. The initial boiling with acid converts polyphosphates to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidizing reagents. In the presence of interfering metal ions, use a larger excess of phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal ions are present, the extraction method may be used. If noticeable amounts of color or organic matter are present, it may be necessary to evaporate the sample, gently ash the residue, and redissolve in acid. The ashing may be carried out in silica, porcelain, or platinum crucibles that have been boiled for several hours in 6N HCl. The presence of excessive amounts of organic matter may necessitate digestion before use of the extraction procedure.

Minimum detectable concentration: Dissolved or total concentrations of iron as low as 10 µg/L can be determined with a spectrophotometer using cells with a 5 cm or longer light path. Carry a blank through the entire procedure to allow for correction.

3. Materials:

Apparatus:

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

-Acid washed glassware: Wash all glassware with conc hydrochloric acid (HCl) and rinse with reagent water before use to remove deposits of iron oxide.

-Volumetric flasks

-Pipettes

Solutions:

Store reagents in glass-stoppered bottles. The HCl and ammonium acetate solutions are stable indefinitely if tightly stoppered. The hydroxylamine, phenanthroline, and stock iron solutions are stable for several months. The standard iron solutions are not stable; prepare daily as needed by diluting the stock solution. Visual standards in nessler tubes are stable for several months if sealed and protected from light.

-Stock iron solution:

Use metal (1) or salt (2) for preparing the stock solution. 1) Use electrolytic iron wire, or "iron wire for standardizing," to prepare the solution. If necessary, clean wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200.0 mg wire and place in a 1000-mL volumetric flask. Dissolve in 20 mL 6N sulfuric acid (H_2SO_4) and dilute to mark with water; 1.00 mL= 200 µg Fe^{2+})

-If ferrous ammonium sulfate is preferred, slowly add 20 mL conc H_2SO_4 to 50 mL water and dissolve 1.404 g $Fe(NH_4)_2(SO_4)_2 .6H_2O$. Slowly add potassium permanganate until a faint pink color persists. Add the last few milliliters of the solution dropwise. Approximately 50 mL of the potassium permanganate will be required. Dilute to 1000 mL with water and mix; 1.00 mL=200 µg Fe

- Standard iron solutions: Prepare daily for use.

Pipet 50.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL=10.0 µg Fe

Pipet 5.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL=1.00 µg Fe

- Hydroxylamine solution:

Dissolve 10 g $NH_2OH.HCl$ in 100 mL water.

- Phenanthroline solution:

Dissolve 100 mg 1,10-phenanthroline monohydrate, $C_{12}H_8N_2.H_2O$, in 100 mL water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops conc HCl are added to the water. (NOTE: One milliliter of this reagent is sufficient for no more than 100 µg Fe)

4. Procedure:

-Pipette 2,6,10,20,40,50 ml standart iron solution into 100 ml volumetreic flasks.

-Add 1 ml Hydroxylamine solution to each flask.

-Add 25 ml Phenanthroline solution, make up to the volüme, and mix well.

-Prepare a blank solution using distilled water by adding the same reagents (Steps 2 and 3) except the standart iron solution.

-Prepare your unknown sample, by adding the same reagents ((Steps 2 and 3)

-Wait for a few minutes fort he color development.

-Measure the absorbance of each solution at 510 nm using the blank solution.

-Plot these results as absorbance versus concentration to obtain the calibration curve.

-Measure the absorbance of the unknown sample and determine its concentration using the calibration curve.

5. Calculations

Standart Fe solution (ml)	Absorbance at 510 nm
2	0,041
6	0,119
10	0,196
20	0,394
40	0,774
50	0,935
Unknown Sample	

Calculate the Fe(II) concentarion of the sample.