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# **BASIC LABORATORY SAFETY**

## **Safety in the Laboratory**

**Purpose:** Faulty technique is one of the chief causes of accidents and, because it involves the human element, is one of the most difficult to cope with. The purpose of this discussion is to help the student understand proper laboratory safety, to increase his awareness of the possible risks or hazards involved with laboratory work and to realize the laboratory is generally a safe place to work if safety guidelines are properly followed.

## **Standard Operating Procedures**

### 🡺General Personal Safety

1. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in areas where specimens are handled.
2. Food and drink are not stored in refrigerators, freezers, cabinets, or on shelves, countertops, or bench tops where blood or other potentially infectious materials are stored or in other areas of possible contamination.
3. Long hair, ties, scarves and earrings should be secured.
4. Keep pens and pencils OUT OF YOUR MOUTH!!
5. Appropriate Personal Protective Equipment (PPE) will be used where indicated
   * **Lab coats** or disposable aprons should be worn in the lab to protect you and your clothing from contamination. Lab coats should not be worn outside the laboratory.
   * **Lab footwear** should consist of normal closed shoes to protect all areas of the foot from possible puncture from sharp objects and/or broken glass and from contamination from corrosive reagents and/or infectious materials.
   * **Gloves** should be worn for handling blood and body fluid specimens, touching the mucous membranes or non-intact skin of patients, touching items or surfaces soiled with blood or body fluid, and for performing venipunctures and other vascular access procedures. Cuts and abrasionsshould be kept bandaged in addition to wearing gloves when handling biohazardous materials.
   * **Protective eyewear** and/or masks may need to be worn when contact with hazardous aerosols; caustic chemicals and/or reagents are anticipated.
6. **NEVER MOUTH PIPETTE!!** Mechanical pipetting devices must be used for pipetting all liquids.
7. Frequent hand washing is an important safety precaution, which should be practiced after contact with patients and laboratory specimens. Proper hand washing techniques include soap, running water and 10-15seconds of friction or scrubbing action. Hands should be dried and the paper towel used to turn the faucets off.

* **Hands are washed:**
  + After completion of work and before leaving the laboratory.
  + After removing gloves.
  + Before eating, drinking, smoking, applying cosmetics, changing contact lenses or using lavatory facilities.
  + Before all other activities which entail hand contact with mucous membranes or breaks in the skin.
  + Immediately after accidental skin contact with blood or other potentially infectious materials.
  + Between patient contact and before invasive procedures.

1. Laboratory work surfaces must be disinfected daily and after a spill of blood or body fluid with a 1:10 dilution of Clorox in water.

### **Eye Safety**

1. Know where the nearest Eye wash station is located and how to operate It.
2. Eye goggles should be worn:

* When working with certain caustic reagents and/or solvents, or concentrated acids and bases.
* When performing procedures that are likely to generate droplets/aerosols of blood or other body fluid.
* When working with reagents under pressure.
* When working in close proximity to ultra-violet radiation (light).

1. Wearing contact lenses in the laboratory is discouraged and requires extra precaution if worn. Gases and vapors can be concentrated under the lenses and cause permanent eye damage. Furthermore, in the event of a chemical splash into an eye, it is often nearly impossible to remove the contact lens to irrigate the eye because of involuntary spasm of the eyelid. Persons who must wear contact lenses should inform their supervisor to determine which procedures would require wearing no-vent goggles.

## **Safe Handling of Biologically Hazardous Material**

## **When working in the laboratory:**

* Wear protective closing (lab coat, gloves. If you have acut/abrasion, also wear a band-aid.
* Avoid spillage and aerosol formation.
* Hands should be washed immediately and thoroughly if contaminated with blood or other body fluids.
* Gloves should be removed before handling a telephone, computer keyboard, etc., and must NOT be worn outside the immediate work area. Hands should always be washed immediately after gloves are removed.
* You should wash your hands after completing laboratory activities and before leaving the area. All protective clothing should be removed prior to leaving the lab.
* All biohazardous material should be discarded in a biohazard bag to be autoclaved.
* All counter and table tops should be disinfected with a proper disinfecting solution:

## **Chemical and Gas Safety**

To provide a safe working environment, all personnel should be aware of potentially hazardous materials and the proper way of handling this material. Avoid unnecessary exposure to chemicals. Occupational Safety and Health Administration (OSHA) requires any necessary information in the form of MATERIAL SAFETY DATASHEETS (MSDS) concerning the handling of hazardous materials to be available to all laboratory personnel, so that they may achieve and maintain safe working conditions.

* **Flammable (Red)**
* **Instability (Yellow)**
* **Health (Blue)**
* **Special Notice (White)**
* **NFPA Chemical Hazard Sign**

## **Toxic and Corrosive Materials (acids and alkali):**

* To avoid dangerous splatter, **ALWAYS ADD ACID TO WATER!**
* Toxic materials should be labeled with special tape when used in compounded reagents and stored in separate containers. These materials should be handled carefully and kept in the hood during preparation.
* Acids and alkali should be carried by means of special protective carriers when transported.
* Acid and alkali spills should be covered and neutralized by using the material from the ‘spill bucket’. All material, spill and compound, should be swept up and placed in a plastic bucket for proper disposal.
* In case of spillage, wash all exposed human tissue (including eyes) generously with water and notify your supervisor for proper reporting of the incident.

## **Carcinogens**

* All laboratory chemicals identified as carcinogens must be labeled **CARCINOGEN**.
* When working with these substances, protective clothing and gloves should be worn.

## **Flammable Compounds**

* All flammable reagents should be kept in the flammable storage facilities (closet or refrigerator) at all times when not in use.
* Any solutions compounded from these reagents should be labeled as flammable.
* Flammable substances should be handled in areas free of ignition sources.
* Flammable substances should never be heated using an open flame.
* Ventilation is one of the most effective ways to prevent accumulation of explosive levels of flammable vapors. An exhaust hood should be used whenever appreciable quantities of flammables are handled.
* Flammable compounds should be placed in proper receptacle for disposal.

## **Ether Precautions (flammable compound)**

* These compounds tend to react with oxygen to form explosive peroxides. When ether containers are opened they are to be dated and all material remaining after six (6) months must be disposed of immediately.
* Disposal of ether compounds is through the Hazardous Materials Office.
* Ether compounds will be stored in an explosion-proof refrigerator. (boiling point of ether is approximately room temperature)

## **Compressed Gases**

* The storage of all compressed gases shall be in containers designed, constructed, tested and maintained in accordance with the U.S. Department of Transportation Specifications and Regulations.
* In the laboratory, gas containers are to be limited to the number of containers in use at any time. Low pressure (LP) gases shall also be limited to the smallest size container.
* Containers shall be securely strapped, chained or secured in a cylinder stand so they cannot fall.
* Oxidizing gases should be separated from flammable gasses.

## **Radiation Safety**

* No eating, drinking, smoking permitted!
* Radioactive material should be labeled as radioactive and stored in a proper container so as to prevent spillage or leakage.
* These materials must be handled carefully. Remember: **the amount of radiation exposure decreases with distance.**
* Radioactive spills should be absorbed with absorbent toweling. The area should be cleaned with soap and water and then decontaminated with a product such as ‘count-off’. The area of the spill is then monitored for any residual radioactivity. If the area is not decontaminated, the above regimen is repeated and re-monitored.
* In the case of a radioactive spill in a high traffic area, the area will be ‘roped off’ until proper decontamination has been achieved.
* In the case of a major radioactive spill, all personnel in the area must be notified. The appropriate safety officer must be notified and all attempts to keep contamination at a minimum must be used.

## Fire Safety

* For burning combustible materials (wood, paper, clothing, trash). **GREEN TRIANGLE WITHTHE LETTER ‘A’**, uses water or an all-purpose dry chemical.
* For burning liquids: **RED SQUARE WITH THE LETTER‘B’,** uses foam, a dry chemical or carbon dioxide.
* For electrical fires: **BLUE CIRCLE WITH THE LETTER ‘C’** uses non-conducting extinguishing agents (carbon dioxide or a dry chemical).

## **R A C E**

1. **R**escue those in danger
2. **A**larm
3. Activate the fire pull station
4. Notify switchboard operator of the location, your name and thetype of fire, if known
5. **C**ontain the fire by closing all doors and windows
6. **E**xtinguish the fire, if possible. Do not re-enter a room that has alreadybeen closed.

## **General Procedures and Equipment**

* Cracked or chipped glassware should not be used.
* Centrifuges should not be used without the covers completely closed.
* When removing tops from evacuated test tubes, care must be taken to prevent aerosol formation.

## **In Case of Accidents**

* Bleed wound.
* Wash wound thoroughly with soap.
* Notify the supervisor of the incident and report to Student Health with an incident report form.

# **Meaning of Water Quality Analysis**

Water quality standards are put in place to ensure the efficient use of water for a designated purpose. Water quality analysis is to measure the required parameters of water, following standard methods, to check whether they are in accordance with the standard.

## **Requirement of Water Quality Analysis:**

Water quality analysis is required mainly for monitoring purpose. Some importance of such assessment includes:

* To check whether the water quality is in compliance with the standards, and hence, suitable or not for the designated use.
* To monitor the efficiency of a system, working for water quality maintenance.
* To check whether up gradation / change of an existing system is required and to decide what changes should take place.
* To monitor whether water quality is in compliance with rules and regulations.



## **Sampling of Water for Analysis:**

A common cause of error in water quality analysis is improper sampling. The results of a water quality analysis of a sample show only what is in the sample. For the results to be meaningful, the sample must be representative i.e., it must contain essentially the same constituents as the body of water from which it was taken.

The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and yet large enough for analytical purposes while still accurately representing the material being sampled.

The complexity of water quality as a subject is reflected in the many types of measurements of water quality indicators. The most accurate measurements of water quality are made on-site, because water exists in equilibrium with its surroundings. Measurements commonly made on-site and in direct contact with the water source in question include temperature,' pH, dissolved oxygen, electric conductivity, etc. More complex measurements are often made in a laboratory requiring a water sample to be collected, preserved, transported, and analyzed at another location.

## **Requirements for Sampling:**

* Meet the requirements of the sampling program.
* Handle the sample carefully so that it does not deteriorate or become contaminated or compromised before it is analyzed.
* Ensure sampling all equipment are clean and quality assured before use.
* Use sample containers that are clean and free of contaminants.
* Rinse the bag/bottle at least twice with the sample water prior to filling and closing.
* Fill bag/bottle as full as possible. Half-filling leaves more room for oxygen which will promote degradation of your sample.
* If sampling a body of running water, point the mouth of the bag upstream and your hands downstream to avoid contamination.
* If sampling from a water faucet, run the faucet for 1 minute before obtaining a sample.
* Make records of every sample collected and identify every bottle e.g., take notes and photographs, fill out tags, etc.
* Place the sample into appropriate, labeled containers.
* All samples must be preserved as soon as practically possible.

## **Sample Collection bottles, Size and Materials:**

The methods that will be followed will determine the type of bottles used. For example, samples for metals’ analyses are usually collected in plastic bottles, while analyses for volatile organics and pesticides are collected in glass containers. Bottles used to collect samples for bacteria should be sterilized. Certain analysis like volatile organics and radon require vials that are to be filled leaving no head space, which keeps these analytes dissolved in the water, preventing them from escaping into the air. Additionally, some analyses require samples to be collected in amber colored bottles. These darker bottles are for analytes that break-down in sunlight, which helps keep these contaminants from breaking down while in transit to the laboratory for analysis. The size of the container is important to ensure enough samples to run the analysis needed.

## **Sampling water from a tap for microbiological analysis**

* Carefully clean and disinfect the inside and outside of the tap.
* Open the tap and let water flow for 2-3 minutes or until the water temperature has stabilized.
* Turn off the tap and sterilize the spout by heating it with a blow lamp, gas torch or by igniting a piece of cotton wool soaked with methylated spirits until any water in the tap boils.
* Take care not to allow the container to touch the tap.
* Take a water sample with the sample container.

## **Sample Preservation:**

There is usually a delay between the collection and analysis of a sample. The nature of the sample can be changed during this period. Therefore proper preservation is required in the way to laboratory after collection, and in the laboratory up to when analysis starts. Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, or natural waters, is practically impossible as because - complete stability for every constituent never can be achieved.

At best, preservation techniques only retard chemical (especially, hydrolysis of constituents) and biological changes that inevitably continue after sample collection. No single method of preservation is entirely satisfactory; the preservative is chosen with due regard to the determinations to be made.

Commonly used preservation methods are - pH control, chemical addition, the use of amber and opaque bottles, refrigeration, filtration, and freezing.

# **pH IN WATER**

## **1.1 Aim: To Determine the pH of the given water sample.**

## **1.2 Apparatus:**

1. pHmeter 2. Beaker

## **1.3 Reagent:**

• *Potassium chloride (1M):*  Dissolve 74.5 g of KCl in distilled water and makeup

to one litre to get a 1M solution.

• *Buffer pH = 4, 7 and 9:* Dilute the respective ampoules according to the

manufacturer’s instructions.

## **1.4 Principle**

The pHelectrode used in the pHmeasurement is a combined glass electrode. It consists of sensing half-cell and reference half-cell, together form an electrode system. The sensing half-cell is a thin pHsensitive semi permeable membrane, separating two solutions, viz., the outer solution, the sample to be analyzed and the internal solution enclosed inside the glass membrane and has a known pHvalue. An electrical potential is developed inside and another electrical potential is developed outside, the difference in the potential is measured and is given as the pHof the sample.

## **1.5 Sample handling and preservation**

Preservation of sample is not practical. Because biological activity will continue after a sample has been taken, changes may occur during handling and storage. The characteristics of the water sample may change. To reduce the change in samples taken for the determination of pH, keep samples at **4°C**. Do not allow the samples to freeze. Analysis should begin as soon as possible.

The values of pH**,** 0 to a little less than 7 are termed as acidic and the values of pHa little above **7** to **14** are termed as basic. When the concentration of **H+** and **OH-**ions are equal then it is termed as neutral pH.

## **Environmental significance:**

Determination of pHis one of the important objectives in biological treatment of the wastewater. In anaerobic treatment, if the pHgoes below **5** due to excess accumulation of acids, the process is severely affected. Shifting of pH beyond **5** to **10** upsets the aerobic treatment of the wastewater. In these circumstances, the pHis generally adjusted by addition of suitable acid or alkali to optimize the treatment of the wastewater. pH value or range is of immense importance for any chemical reaction. A chemical shall be highly effective at a particular pH. Chemical coagulation, disinfection, water softening and corrosion control are governed by pHadjustment.

|  |  |
| --- | --- |
| Lower value of pHbelow **4** will produce sour taste and higher value above **8.5** a bitter taste. Higher values of pHhasten the scale formation in water heating apparatus and also reduce the germicidal potential of chlorine. |  |
| High pHinduces the formation of tri-halo-methane, which are causing cancer in human beings. | |

## **Precautions**

The following precautions should be observed while performing the experiment:

* Temperature affects the measurement of pHat two points. The first is caused by the change in electrode output at different temperatures. This interference can be controlled by the instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second is the change of pHinherent in the sample at different temperatures. This type of error is sample dependent and cannot be controlled; hence both the pHand temperature at the time of analysis should be noted.
* In general, the glass electrode is not subject to solution interferences like color, high salinity, colloidal matter, oxidants, turbidity or reductants.
* Oil and grease, if present in the electrode layer, should be removed by gentle wiping or detergent washing, followed by rinsing with distilled water, because it could impair the electrode response.
* Before using, allow the electrode to stand in dilute hydrochloric acid solution for at least 2 hours.
* Electrodes used in the pH meter are highly fragile, hence handle it carefully.

## **1.8 Procedure:**

Three major steps are involved in the experiment. They are

1. Preparation of Reagents 2. Calibrating the Instrument 3. Testing of Sample

### **Steps:**

* Perform calibration of the pHmeter using standard pHsolutions. The calibration procedure would depend on the pHrange of interest.
* In a clean dry **100 mL** beaker take the water sample and place it in a magnetic stirrer, insert the teflon coated stirring bar and stir well.
* Now place the electrode in the beaker containing the water sample and check for the reading in the pHmeter. Wait until you get a stable reading.
* Take the electrode from the water sample, wash it with distilled water and then wipe gently with soft tissue.

## **Report:**

|  |  |  |
| --- | --- | --- |
| S.No | Sample Name | pH Value |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

# **2. CHLORIDES**

## **2.1 Aim:** To determine the amount of chloride present in the given samples

## **2.2 Apparatus:**

|  |  |  |
| --- | --- | --- |
| 1. Burette 2. Measuring cylinder | 1. Dropper 2. Stirrer | 1. Beaker |

## **2.3 Reagents:**

1. 0.02 N Sodium Chloride: 1.170g of NaCl (AR grade dried at 800C for 1 hour) is dissolved in double distilled water and made to one liter.
2. 0.02N Silver Nitrate: 3.40g of Silver nitrate is dissolved in double distilled water and made up to one litre. This is to be standardized against the standard NaCl Sodium solution and stored in amber (brown) colored bottle, away from light.
3. Potassium Chromate Indicator: 5% aqueous solution of pure K2CrO4.

## **2.4 Principle: (Mohr’s Method)**

This method determines the chloride ion concentration of a solution by titration with silver nitrate. As the silver nitrate solution is slowly added, a precipitate of silver chloride forms.

**Ag+(aq) + Cl–(aq) → AgCl(s)**

The end point of the titration occurs when all the chloride ions are precipitated. Then additional silver ions react with the chromate ions of the indicator, potassium chromate, to form a red-brown precipitate of silver chromate.

**2Ag+(aq) + CrO42–(aq)→ Ag2CrO4(s)**

This method can be used to determine the chloride ion concentration of water samples from many sources such as seawater, stream water, river water and estuary water. The pH of the sample solutions should be between 6.5 and 10. If the solutions are acidic, the gravimetric method or Volhard’s method should be used.

The end point of titration cannot be detected visually unless an indicator capable of demonstrating the presence of excess Ag+ is present. The indicator normally used is potassium chromate, which supplies chromate ions. As the concentration of CI- ions becomes exhausted, the silver ion concentration increases and a reddish brown precipitate of silver chromate is formed.

**2Ag++CrO42- = Ag2CrO4****(reddish brown precipitate)**

This is taken as evidence that all chloride has been precipitated. Since an excess Ag+ is needed to produce a visible amount of Ag2CrO4, the indicator error is subtracted from all titrations.

The indicator error or blank varies somewhat with the ability of individuals to detect a noticeable color change. The usual range is 0.2 to 0.4 mL of titrant. An error of 0.2 mL will be used in the class.

## **2.5 Sample Handling and Preservation**

Preservation of sample is not practical. Because biological activity will continue after a sample has been taken, changes may occur during handling and storage.

If Analysis is to be carried out within two hours of collection, cool storage is not necessary. If analysis cannot be started with in the two hours of sample collection to reduce the change in sample, keep all samples at 400 C.

Do not allow samples to freeze. Do not open sample bottle before analysis. Begin analysis within six hours of sample collection

## **2.5 Procedure:**

1. Take 5 ml of sample in porcelin dish and dilute it to about 25 ml with distilled water.
2. Add 5 to 6 drops of K2CrO4.
3. Titrate with standard AgNO3 solution with stirring till the first brick red tinge appears.
4. Note down the volume of AgNO3 solution used from burette.

Chloride is invariably present in small amounts in almost all natural waters and its content goes up appreciably with increasing salinity. The estimation of chloride may be carried out by Mohr’s method, when the electrical conductivity of water sample is greater than one ds/m at 250C.

For public health, chlorides up to 250 mg/liter are not harmful but increases of chlorides beyond this are indication of organic pollution.

## **2.6 Precautions:**

1. A uniform sample size must be used, preferably 100 ml (or 50 mL), so that ionic concentrations needed to indicate the end point will be constant.
2. The pH must be in the range of 7 to 8 because Ag+ is precipitated as AgOH at high pH levels and the CrO4-2 is converted to Cr2O7-2 at low pH levels,
3. A definite amount of indicator must be used to provide a certain concentration of CrO4; otherwise Ag2CrO4 may form too soon or not soon enough.
4. The chromate solution needs to be prepared and used with care as chromate is a known carcinogen.
5. Silver nitrate solution causes staining of skin and fabric (chemical burns). Any spills should be rinsed with water immediately.

## **Calculations:**

# **OIL AND GREASE**

## **3.1 Aim:** To determine the percentage of Oil and grease in the given samples

## **3.2 Apparatus and equipment**

* + 1. Separatory funnel, 1L with TFE (Teflon) stopcock
    2. Distilling flask, 125mL
    3. Water bath
    4. Filter paper, 110mm dia. (Whatman No. 40 or equivalent).
    5. Weighing balance

## **3.3 Reagents and standards**

1. Hydrochloric acid: HCl (1+1)
2. n-hexane
3. Petroleum ether (BP 40°C-60°C) or Hexane
4. Anhydrous sodium sulphate-Na2SO4

The solvent should leave no measurable residue on evaporation; distill if necessary. Petroleum ether40°C/60°C or hexane can also be used. Plastic tubes should not be used to transfer solvent between containers.

## **3.4 Principle**

## **Partition-gravimetric method**

Dissolved or emulsified oil and grease is extracted from water by intimate contact with n-hexane, petroleum ether (40°C/60°C) or hexane. Unsaturated fats and fatty acids oxidize readily hence precautions regarding temperature to solvent vapour displacement are included in the procedure.

Oil and grease is any material recovered as a substance soluble in petroleum ether, hexane or n-hexane. It includes other materials extracted by the solvent from an acidified sample such as sulphur compounds, certain organic dyes and chlorophyll. Oil and grease are defined by the method used for their determination. The oil and grease content of domestic industrial wastes and of sludges, is an important consideration in the handling and treatment of these materials for ultimate disposal. When treated effluents are discharged in water body, it leads to environmental degradation. Hydrocarbons, esters, oils, fats, waxes and high molecular weight fatty acids are the major materials dissolved by hexane. All these materials have a ‘greasy feel’.

## **3.5 Sample collection, preservation and storage**

Collect separate sample for oil and grease and do not subdivide in the laboratory. Samples collected at different intervals of time may be examined individually for knowing average concentration of oiland grease. The glass bottle container should be rinsed with the solvent to remove contaminants adhered to the side walls.

### **3.6 Procedure**

* Collect about 1L sample and mark sample level in bottle for later determination of sample volume. Acidify to pH 2 or lower; generally, 5mL HCl (1+1) is sufficient. Transfer to a separatory funnel. Carefully rinse sample bottle with 30mL n-hexane and add the solvent washings to separatory funnel.
* Preferably shake vigorously for 2 min. However, if it is suspected for a stable emulsion, shake gently for 5 to 10 min.
* Let the layers separate. Drain solvent layer through a funnel containing solvent-moisten edfilter paper and 10g Na2SO4 into a clean, tared distilling flask. If a clear solvent layer cannot be obtained and emulsion exists, centrifuge the solvent and emulsion. Transfer centrifuged material to a separating funnel and drain solvent layer through a funnel with a prerinsed filter paper and 10 g Na2SO4.
* Extract twice more with 30mL solvent each but first rinse sample container with each solvent portion. Combine extracts in tared distilling flask and wash filter paper with an additional 10to 20mL solvent.
* Distill solvent from distilling flask in a water bath at 70°C for solvent recovery. Place flask on a water bath at 70°C for 15 min and draw air through it with applied vacuum for the final1min after the solvent has evaporated. If the residue contains visible water, add 2mL acetone, evaporate on a water-bath and repeat the addition and evaporation until all visible water has been removed. Cool in a Desiccator for 30 min and weigh.

## **3.7 Calculations**

Total gain in weight A, of tared flask and less calculated residue B, from solvent blank is the amount of oil and grease in the sample.

Mg/L, Oil and grease n = (A –B) X 1000 / mL sample

Along with the results, mention the solvent used for extraction.

# **4. TOTAL SOLIDS, DISSOLVED SOLIDS AND SUSPENDED SOLIDS IN WATER**

## **4.1 Aim:** Determination of Total Solids, Dissolved Solids and Suspended Solids in Water

## **4.2 Apparatus:**

|  |  |  |
| --- | --- | --- |
| 1. Balance 2. Beaker | 1. Measuring Cylinder 2. Filter paper | 1. Funnel 2. Dropper |

## **4.3 Reagents:**

There is no reagents are to be found

## **4.4 Principle:**

The measurement of solids is by means of the gravimetric procedure. The various forms of solids are determined by weighing after the appropriate handling procedures. The total solids concentration of a sample can be found directly by weighing the sample before and after drying at 103°C. However, the remaining forms, TDS and TSS require filtration of the sample. For liquid samples, all these solids levels are reported in mg/L.

A rapid assessment of the dissolved solids content of water can be obtained by specific conductance measurements. Such measurement indicates the capacity of a sample to carry an electric current which in turn is related to the concentration of ionized substances in the water. Most dissolved inorganic substances in water are in ionized form and so contribute to the specific conductance. Although the nature of the various ions, their relative concentrations, and the ionic strength of the water affect this measurement, such measurement can give practical estimate of the dissolved mineral content of water. The TDS content can be approximated by multiplying the specific conductance in micro-Siemens per cm (μS/cm) by an empirical factor varying from 0.55 to 0.90 depending on the chemical composition of the TDS.

### **4.4.1 Theory:**

Environmental engineering is concerned with the solid material in a wide range of natural waters and wastewaters. The usual definition of solids (referred to as "total solids") is the matter that remains as residue upon evaporation at 103~105°C. The various components of "total solids" can be simplified as follows

Total Solids (TS) are the total of all solids in a water sample. They include the total suspended solids and total dissolved solids. Total Suspended Solids (TSS) is the amount of filterable solids in a water sample. Samples are filtered through a glass fiber filter. The filters are dried and weighed to determine the amount of total suspended solids in mg/l of sample. Total Dissolved Solids (TDS) are those solids that pass through a filter with a pore size of 2.0 micron (1/1000000th of a meter, Also known as a Micrometer) or smaller. They are said to be non-filterable. After filtration the filtrate (liquid) is dried and the remaining residue is weighed and calculated as mg/l of Total Dissolved Solids.



Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations

Although the waste water or sewage normally contains 99.9 percent of water and only 0.1 percent of solids, but it is the solids that have the nuisance value. The amount of solids in wastewater is frequently used to describe the strength of the water. The more solids present in a particular wastewater, the stronger that wastewater will be. The environmental impacts of solids in all forms have detrimental effects on quality since they cause putrefaction problems. If the solids in wastewater are mostly organic, the impact on a treatment plant is greater than if the solids are mostly inorganic.

In the realm of municipal wastewater, suspended solids analysis is by far the most important gravimetric method. It is used to evaluate the strength of the raw wastewater as well as the overall efficiency of treatment. Furthermore, most waste water treatment plants (WWTP’s) have effluent standards of 10 to 30 mg/L suspended solids which may be legally enforceable. As was the case with municipal wastewater, suspended solids analysis is useful as a means of assessing the strength of industrial wastewaters and the efficiency of industrial wastewater treatment.



Dissolved minerals, gases and organic constituents may produce aesthetically displeasing color, taste and odor. Some dissolved organic chemicals may deplete the dissolved oxygen in the receiving waters and some may be inert to biological oxidation, yet others have been identified as carcinogens. Water with higher solids content often has a laxative and sometimes the reverse effect upon people whose bodies are not adjusted to them. Estimation of total dissolved solids is useful to determine whether the water is suitable for drinking purpose, agriculture and industrial purpose. Suspended material is aesthetically displeasing and provides adsorption sites for chemical and biological agents. Suspended organic solids which are degraded an aerobically may release obnoxious odors. Biologically active suspended solids may include disease causing organisms as well as organisms such as toxic producing strains of algae. The suspended solids parameter is used to measure the quality of wastewater influent and effluent. Suspended solids determination is extremely valuable in the analysis of polluted waters. Suspended solids exclude light, thus reducing the growth of oxygen producing plants. High concentration of dissolved solids about 3000 mg/L may also produce distress in livestock. In industries, the use of water with high amount of dissolved solids may lead to scaling in boilers, corrosion and degraded quality of the product.

## **4.5 Sample handling and preservation:**

Preservation of sample is not practical. Because biological activity will continue after a sample has been taken, changes may occur during handling and storage. Both the characteristics and the amount of solids may change. To reduce this change in samples taken for solids determinations, keep all samples at 4° C. Do not allow samples to freeze. Analysis should begin as soon as possible.

## **4.6 Precautions:**

The following precautions should be observed while performing the experiment:

* Water or Wastewater samples which contain high concentrations of calcium, chloride, magnesium or sulphate can rapidly absorb moisture from the air. Such samples may need to be dried for a longer period of time, cooled under proper desiccation and weighed rapidly in order to achieve a reasonable constant weight. We should be aware prolonged drying may result in loss of constituents, particularly nitrates and chlorides.
* 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.
* Floating oil and grease, if present, should be included in the sample and dispersed by a blender device before sub-sampling.
* Volume of sample should be adjusted to have residue left after drying as 100 to 200mg. It is mainly to prevent large amount of residue in entrapping water during evaporation.
* Highly mineralized water containing significant concentration of calcium, magnesium, chloride, and/or sulphate may be hygroscopic. Hence prolonged drying, desiccation and rapid weighing.
* We should be aware prolonged drying may result in loss of constituents, particularly nitrates and chlorides.

## **4.7 Procedure:**

### **4.7.1 Measurement of Total Solids (TS)**

* 1. Take a clear dry glass beaker (which was kept at 103°C in an oven for 1 hour) of 150ml. capacity and put appropriate identification mark on it. Weight the beaker and note the weight.
  2. Pour 100ml. of the thoroughly mixed sample, measured by the measuring cylinder, in the beaker.
  3. Place the beaker in an oven maintained at 103°C for 24hours. After 24 hours, cool the beaker and weight. Find out the weight of solids in the beaker by subtracting the weight of the clean beaker determined in step (1)
  4. Calculate total solids (TS) as follows:

### **4.7.2 Measurement of Total Dissolved Solids (TDS)**

1. Same as above (step 1 of total solids).
2. Take a 100 ml. of sample and filter it through a double layered filter paper and collect the filtrate in a beaker.
3. The repeat the same procedure as in steps (3) and (4) of the total solids determination and determine the dissolved solids contents as follows:

## **4.8 Calculation:**

**Total solids, TS (mg/l) =** mg of solids in the beaker x 1000 / (volume of sample)

**Total Dissolved Solids, TDS (mg/l) =** mg of solids in the beaker x1000 /(volume of sample)

**Total Suspended Solids, TSS (mg/l) =** TS (mg/l) – TDS (mg/l)

# **5 SULPHIDE**

## **5.1 Aim**: To determine the amount of sulphide present in the sample by titrimetric method.

## **5.2 Apparatus**

1. Burette
2. Pipette
3. Erlenmeyer flask

## **5.3 Reagents**

1. Hydrochloric acid
2. Standard iodine solution (0.025N)
3. Standard sodium thiosulphate solution (0.025N)
4. Starch solution

## **5.4 Principle**

Sulphides often occur in ground water especially in hot springs, in wastewater and polluted waters. Hydrogen sulphide escaping into the air from sulphide containing wastewater causes odour nuisance. It is highly toxic and cause corrosion of sewers and pipes. Sulphides include H2S and HS – and acid soluble metallic sulphides present in the suspended matter. Iodine reacts with sulphide in acid solution, oxidising it to sulphur; a titration based on this reaction is an accurate method for determining sulphides at concentration above 1mg/L if interferences are absent and if loss of H2S is avoided.

## **5.5 Sample Handling**

### 5.5.1 Container Description:

250 or 500 mL polyethylene bottle.

### 5.5.2 Handling and Treatment:

Use unfiltered sample to rinse bottle. Addition zinc acetate at a rate of 2 grams of zinc acetate to a liter of water will fix the sample for several days

## **5.6 Procedure**

1. Measure from a burette 10mL of iodine into a 500 mL flask.
2. Add distilled water and bring the volume to 20 mL.
3. Add 2 mL of 6N HCl.
4. Pipette 200 mL sample into the flask, discharging the sample under the surface of solution.
5. If the iodine colour disappears, add more iodine so that the colour remains.
6. Titrate with sodium thiosulphate solution, adding a few drops of starch solution, as the end point is approached and continuing until the blue color disappears.

### **5.5.1 Observation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample number** | **Volume of Iodine solution used** | **Volume of sodium thiosulphate solution used** | **Volume of sample** | **mg/L**  **Sulphide** |
|  |  |  |  |  |
|  |  |  |  |  |

## **5.8 Calculation**

|  |  |
| --- | --- |
| mg/L sulphide = |  |
|  |

Where, a = mL 0.025 N iodine used,

b = mL 0.025 N sodium thiosulphate solution used.

# **6 DISSOLVED OXYGEN**

## 6.1 **Aim**: To Determine the D.O of given samples

## **6.2 Materials:**

1. Oxygen meter with dissolved oxygen probe.
2. Electrical stirrer
3. 5% Sodium Sulphite Solution

## **6.3 Materials and Reagents:**

1. BOD battles (100 – 300 ml)
2. Manganoussulphate solution: Dissolve 100g of manganousSulphate in 200 ml of previously boiled water and filter the solution.
3. Alkaline potassium iodide solution: Weigh 50g of potassium iodide and 100g of potassium hydroxide. Dissolve the chemicals in 200 ml of previously boiled distilled water.
4. Sodium thiosulphate solution (0.025N): Dissolve 6.0205 of of sodium thiosulphate, in 1 litre previously boiled distilled water and add a pallet of NaOH as a preservative. Keep it in coloujred bottle.
5. Starch indicator: Dissolve 1g starch in 100 ml warm distilled water and add a few drops of toluene as preservative.
6. Concentrated Sulphuric acid: Sp. Gravity 1.84

## **6.4 Theory:**

Dissolved oxygen in water is an index of physical and biological processes going on, non-polluted surface waters are generally saturated with dissolved oxygen. There are two main sources of dissolved oxygen in water.

1. Diffusion from air / absorption from air
2. Photosynthetic activity within water by vegetation etc.

Diffusion of air from air to water is a physical phenomenon and is influenced by factors which affect the oxygen solubility like temperature, water movement and salinity etc. Photosynthetic activity within water is a biological phenomenon carriedout by autotrophs (mainly phytoplanktons in water) and depends upon autotroph population, light conditions, and available gases etc.

Oxygen is considered to be a limiting factor, especially in lakes and in waters with a heavy load of organic material. Organisms have specific oxygen requirements. Low dissolved oxygen may prove fatal for many organisms for their survival.

The dissolved oxygen in water can be determined by the following two methods.

1. Electrode or Oxygen mater method, and
2. Iodometric method (Winkler’s method)

Oxygen meter method: This method is convenient, quick or reasonably accurate.

## **6.5 Method:**

Read the operation manual carefully and adjust the instrument accordingly. Dip the D.O probe in 5% Sodium Sulphate Solution with constant stirring. Set meter to zero mark. Now dip the Do probe in water sample being constantly stirred, and record the dissolved oxygen in mg/ litre from the scale.

## **6.6 Principle:**

WINLER’S METHOD: Oxygen combines with Mn(OH)2 and forms higher hydroxides which on subsequent acidification in the presence of iodide, liberate iodine in an amount equivalent to the original dissolved oxygen content of the sample. The I2 is then determined by titration with NO2S2O3.

## **6.7 Procedure**

### **Steps:**

1. Take a glass stoppered BOD bottle of known volume (100-300ml) and fill it with sample avoiding any bubbling. No air should be trapped in bottle after the stoper is placed.
2. Open the bottle and pour in each 1 ml of manganoussulphate and alkaline potassium iodide solution using separate pipettes. If the volume of sample is over 200 ml add 2 ml of each reagent instead of 1 ml
3. A precipitate will appear place the stopper and shake the bottle thoroughly. Sample at this stage can be stored for a few days, if required.
4. Add 2 ml of sulphuric acid to dissolve the precipitate, stake throughly.
5. Transfer gently (avoiding bubbling) whole content (or) a known part of it, in a conical flask. Put a few drops of starch indicator. Titrate against sodium thiosulphate solution and note the end point when initial blue color disappears.

## **6.8 Calculation:**

1. It whole content is used for titration.



1. It a fraction of the content is used for titration



Where D:O = Dissolved Oxygen

= Volume of titrant (ml)

= Volume of sampling bottle after placing the stopper (in ml)

= Volume of manganoussulphate + potassium iodide added (ml)

= Volume of fraction of the content used for titration (ml)

= Normality of titrant (0.025). The equivalent weight of oxygen is g.

*NOTE:* 1. to obtain the value of DO in ml/litre divide the DO in mg/litre by 1.43.

2. The original winker’s method is modified by adding sodium azide in alkaline potassium iodide solution. This avoids the interference due to organic matter and chlorides present in the sample. The other reagents and procedure are the same as described above.

Preparation of alkaline KI solution for modified Winkler’s method: Dissolve separately 350g KOH and 75 g KI in distilled water, mix the two and make the volume 500 ml with distilled water. Dissolve separately 5g of sodium azide (NaN3) in 20 ml of distilled water mix alkaline iodide and sodium azide solutions.

## **6.9 Table:**

|  |  |  |
| --- | --- | --- |
| S.No | Sample Name | D.O Value |
|  |  |  |
|  |  |  |
|  |  |  |

# **7 PHENOLS**

## **7.1 Aim:** To determine the amount of phenol or phenolic compounds present in the samples

## **7.2 Apparatus and equipment**

* 1. Distillation assembly: All glass consisting of 1 L Pyrex distilling apparatus with Graham condenser.
  2. Spectrophotometer for use at 460 nm
  3. pH meter
  4. Separatory funnels: 1000 mL Squibb form, with ground glass stoppers and Teflon stopcocks
  5. Beakers: 1L.

## **7.3 Reagent and standards**

1. Phosphoric acid (1+9): Dilute 10 mL 5% H3PO4 to 100 mL with distilled water.

2. Methyl orange indicator: Dissolve 0.5 g methyl orange in one litre distilled water.

3. Sulphuric acid 1N: Dilute 28 mL of conc. H2SO4 to 1 L with distilled water.

4. Sodium chloride: NaCl, solid crystals.

5. Chloroform or ethyl ether: CHCl3, AR grade.

6. Sodium hydroxide, 2.5N: Dissolve 10 g NaOH in 100 mL distilled water.

7. Stock phenol solution: Dissolve 1 g phenol in freshly boiled and cooled distilled water and dilute to 1 litre. Standardise the stock phenol solution. 1 mL = 1mg phenol.

8. Intermediate phenol solution: Take 10 mL or appropriate volume of stock phenol solution in 1 litre volumetric flask and dilute to the mark with freshly boiled and cooled distilled water asto get 1 mL = 10 μg phenol.

9. Standard phenol solution: Dilute 50 mL intermediate phenol solution to 500 mL with freshly boiled and cooled distilled water. This solution should be prepared within two hours of use. 1mL = 1 mg phenol.

10. Bromate bromide solution (0.1N): Dissolve 2.784 g anhydrous KBrO3 in water, add 10 g KBrcrystals, dissolve and dilute to 1000 mL with distilled water.

11. Hydrochloric acid: HCl conc.

12. Standard sodium thiosulphate (0.025N): Dissolve 6.205 g Na2S2O3.5H2O in distilled water and dilute to 1000 mL.

13. Starch solution: Dissolve 2 g laboratory grade soluble starch in 100 mL hot distilled water. Prepare this solution daily.

14. Ammonium hydroxide (0.5N): Dilute 35 mL fresh concentrated NH4OH to 1000 mL with distilled water.

15. Phosphate buffer solution: Dissolve 104.5 g K2HPO4 and 72.3 g KH2PO4 in distilled water and dilute to 1 litre, the pH of this solution should be 6.8.

16. 4-Aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in distilled water and dilute to 100 mL. Prepare this solution daily.

17. Potassium ferricyanide solution: Dissolve 8.0g K3Fe (CN)6 in water and dilute to 100 mL. Filter if necessary and store in a brown glass bottle.

18. Sodium sulphate: Anhydrous Na2SO4, granular.

19. Potassium iodide: Kl, solid crystals.

## **7.4 Sample collection, preservation and storage**

After collection of sample, analyse within 4 hours, if not, preserve and store by acidifying to pH 4with H3PO4 under cool environment (5°C). Add 1 g CuSO4.5H2O/L of sample to inhibit biological-degradation. Analyze preserved samples within 24 hours.

## **7.5 Theory:**

The presence of organic compounds in water and wastewater is a matter of increasing concern to the water industry, environmentalists and general public. The impact of such compounds may differ. For example, biologically oxidisable compounds will deplete oxygen in the water body and petroleum products. Oil and grease will adversely affect biological activity. Phenols, detergents and other organic materials may be toxic to phyto and zoo-plankton beyond certain levels. Organic compounds may enter water environment through human waste disposal and industrial discharges.

Phenols are defined as hydroxyl derivatives of benzene, and its condensed nuclei occur in domestic and industrial wastewaters, natural wastes and potable water supplies. Odoriferous and objectionable tasting chlorophenols are formed as a result of chlorination of water containing phenol. Phenols maybe present in raw water owing to the discharge of wastewaters from coke distillation plants, the petrochemical industry and numerous other industries where phenols serve as intermediates. They are also present in municipal waste waters. Presence of phenols may lead to objectionable taste in chlorinated drinking water and hence its monitoring is essential. Phenols can be removed from drinking water by super-chlorination (chlorinedioxide or chloramines treatment) ozonation and activated carbon adsorption.

## **7.6 Chloroform extraction method**

The steam distillable phenols react with 4-aminoantipyrine at a pH of 7.9 in presence of potassium-ferricyanide to form a colored antipyrine dye. The dye is extracted from aqueous solution with chloroform and the intensity is measured at 460 nm. This method is applicable in the concentration range of 1 μg/L to 250 μg/L with a sensitivity of μg/L.

## **7.7 Procedure**

### **7.7.1. Distillation:**

1. Measure 500 mL sample into a beaker, add 50 mL phenol-free distilled water, lower the pH to 4.0 with H3PO4 solution using methyl orange as an indicator. Add 5 mLCuSO4 solution. Transfer to distillation flask and collect 500 mL distillate using measuring cylinder as receiver. If the distillate is turbid repeat the same procedure as above. Omit addition of H3PO4 and CuSO4 if the preserved sample is used.
2. Take 500 mL original sample. Make it acidic with 1 N H2SO4 using methyl orange asan indicator. Transfer into a separating funnel and add 150 g NaCl. Shake with five increment of chloroform, using 40 mL in the first increment and 25 mL in each of the following increments. Transfer the chloroform layer to another separatory funnel and shake with three successive increments of 2.5 N NaOH solution using 4.0 mL in the first increment and 3.0 mL in each of the next two increments. Combine the alkaline extracts. Heat on water bath until the chloroform has been removed. Cool and dilute to500 mL with distilled water and proceed for distillation as in (A).

### **7.7.2 Extraction and colour development:**

* 1. Take 500 mL of the distillate, or a suitable portion containing for more than 50 mg phenol and dilute to 500 mL in 1 litre beaker.
  2. Take 500 mL distilled water blank and a series of 500 mL phenol standards containing 5, 10, 20, 30, 40 and 50 μg phenol, in respective beakers.
  3. Add 12 mL 0.5 N NH4OH solution and adjust the pH of each to 7.9 ± 0.1 with phosphate buffer. About 10 mL phosphate buffer is required. Transfer to 1 litre separating funnel, add3.0 mL 4-aminoantipyrine solution in each separatory funnel, mix well and add 3.0 mL potassium ferricyanide, again mix well and let the color develop for 15 min.
  4. Add 25 mL chloroform in each separatory funnel and shake at least 10 times, let the CHCl3settle again. Filter each CHCl3 extract through filter paper containing 5 g layer of anhydrousNa2SO4.
  5. Collect dried extract clean cells and measure the absorbance of sample and standard against the blank at 460 nm. Plot absorbance against mg phenol concentration and draw a calibration curve. Estimate sample phenol content from photometric reading by using a calibration curve.

## **7.8 Calculations**

Use of calibration curve, μg/L, phenol = [(A / B) x 1000]

Where:

A = μg phenol in sample (estimated from calibration curve)

B = mL original sample

## **7.9 Safety**

Hazardous chemicals like chloroform, phosphoric acid, potassium ferricyanide, phenol, etc. should be handled with required precautions.

# **8. DISSOLVED OXYGEN (DO)** (Drinking water sample & Sewage water)

## **8.1 AIM**: -Todetermine the Dissolved Oxygen (DO) of drinking water sample and sewage water

## **8.2 Solution REQUIRED:-**

1. 0.05N K2Cr2O7 in100.0 mL
2. 0.05N Na2S2O3 in 250.0 mL
3. 10% KI Solution (10.0 g of KI in 100.0 mL water)
4. MnSO4 Solution (36 g MnSO4.5H2O IS Dissolved in 100mL of water)
5. Sodium Azide Solution (20.0g of NaAOH +10.0 g of KI + I g of NaN2 are Mixed and Dissolved in 100.0 mL of water)
6. H3PO4
7. Freshly Prepared Starch Solution

## **8.3 Steps:**

### 8.3.1 STEP-1:- STANDARDISATION of HYPO BY USING K2CR2O7 Solution :-

Pippet out 10.0 mL 0.05N K2Cr2O7 of into a 250 mL Conical flask and add 5.0 mL of Concentrated HCL, 30.0 mL of water, 5.0 mL of 10% KI Solutions are added.

To this content 2.0 mL of Hypo Solution is added through burette. Shake the contents for few min and add 1.0 mL of freshly prepared starch solution and kept this solution in dark for few min...This solution turns into dirty black now titrate this solution against hypo. The end point is the color change from dirty black to blue or colorless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. OF. K2CR2O7 | BURETTE READING | | VOL. of HYPO |
| INITIAL | FINAL |
| 1 | 10.0 | 0 |  |  |
| 2 | 10.0 |  |  |  |
| 3 | 10.0 |  |  |  |

N1V1 = N2V2

### 8.3.2 STEP-2:- ESTIMATION of D.O in water SAMPLE:-

Reagent bottle is cleaned and filled with water sample and it is immediately closed with a lid to prevent entering the atmospheric oxygen into the sample. Now add 5.0 mL of MnSO4 solution with the help of a syringe. In the same way add 5.0 mL of Sodium Azide until a brown precipitate is formed in the bottle. Same solutions are added again to form a complete precipitate in the bottle.

Now add con H2So4 to the precipitate for complete dissolution. Now the dissolved solution is transferred into 250.0 mL conical flask and add 1.0 mL of freshly prepared starch solution and kept this solution. The solution turns into dirty black now titrate this solution against hypo. The end point is the colour change from dirty black to blue or colourless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. of SAMPLE | BURETTE READING | | VOL. of HYPO |
| INITIAL | FINAL |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

N1V1 = N2V2

# **9 BIOLOGICAL OXYGEN DEMAND** of (Drinking Water Sample & Sewage Water)

## **9.1 AIM:-** Determination of Biological Oxygen Demand (BOD)of drinking Water Sample and Sewage Water

## **9.2 Solution REQUIRED:-**

1. 0.05N K2Cr2O7 in 100.0 mL
2. 0.05N Na2S2O3 in 250.0 mL
3. 10% KI Solution (10.0g of KI in 100.0 mL water)
4. MnSO4 Solution (36g MnSO4.5H2O is dissolved in 100mL of distilled water)
5. Sodium Azide Solution (20.0g of NaOH +10.0 g of KI + I g of NaN2 are mixed and dissolved in 100.0 mL of water)
6. H3PO4
7. Freshly Prepared Starch Solution



## **9.3 Theory:-**

BOD is a measure of the activity of Microbial in water bodies. generally it is in natural water bodies are spares because of low quantity of dissolved organic nutrients in water and diluting effect of water increase in microbial population is an indication of increase in organic matter content in water, sewage contamination of water-bodies.

BOD of a water sample is measured by estimating the amount of DO in the water at the start of the experiment and the amount of oxygen present after a specific time. The DO is estimated soon after the collection using one water sample. The other sample is kept in air tight bottles and incubated in dark for s days at 200C or 3 days at 270C . After the incubation period the DO is estimated.

The difference in the amount of DO in the water samples before and after incubation is taken as BOD of the water sample.

## **9.4 Steps**

### 9.4.1 Step-1:- Standardization of Hypo by using K2CR2O7 Solution:-

Pippet out10.0 mL 0.05n K2Cr2O7 of into a 250 mL conical flask and add 5.0 mL of con. HCL, 30.0 mL of water, 5.0 mL of 10% KI solutions are added. To this content 2.0 mL of hypo solution is added through burette. Shake the contents for few min and add 1.0 mL of freshly prepared starch solution and kept this solution in dark for few min..The solution turns into dirty black now titrate this solution against hypo. The end point is the color change from dirty black to blue or colorless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.NO** | **VOL. OF. K2Cr2O7** | **BURETTE READING** | | **VOL. OF HYPO** |
| **INITIAL** | **FINAL** |
| **1** |  |  |  |  |
| **2** |  |  |  |  |
| **3** |  |  |  |  |

N1V1 = N2V2

N1= Normality of K2Cr2O7 , V1= Volume of K2Cr2O7

N2= Normality of HYPO=?, V2 = Volume of HYPO

### 9.4.1 Estimation of D.O for drinking water sample.(before incubation) D1:-

Reagent bottle is cleaned and filled with water sample and it is immediately closed with a lid to prevent entering the atmospheric oxygen into the sample. Now add 5.0 mL of MnSO4 solution with the help of a syringe. in the same way add 5.0 mL of Sodium Azide until a brown precipitate is formed in the bottle. Same solutions are added again to form a complete precipitate in the bottle.

Now add con H2SO4 to the precipitate for complete dissolution. now the dissolved solution is transferred in to a 250.0mL conical flask and add 1.0 mL of freshly prepared starch solution and kept this solution .the solution turns into dirty black now titrate this solution against hypo . The end point is the colour change from dirty black to blue or colourless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. OF SAMPLE(Ml) | BURETTE READING | | VOL. OF . HYPO(Ml) |
| INITIAL | FINAL |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

### 9.4.2 Calculation:-

THE AMOUNT of D.O in PPM = x1000

= ppm

### 9.4.2 Estimation of D.O for Drinking Water Sample. (After Incubation) D2:- (for 3 days at 270c)

Reagent bottle is cleaned and filled with water sample and it is immediately closed with a lid and covered with a parafin tape. Now put this bottle in the incubator **for 3 days at 270c**. After 3 days, add 5.0 mL of MnSO4 solution with the help of the syringe. In the same way add 5.0 mL of Sodium Azide until a brown precipitate is formed in the bottle. Same solutions are added again to form a complete precipitate in the bottle.

Now add con H2SO4 to the precipitate for complete dissolution. Now the dissolved solution is transferred in to a 250.0 mL conical flask and adds 1.0 mL of freshly prepared starch solution and kept this solution. The solution turns into dirty black now titrate this solution against hypo. The end point is the colour change from dirty black to blue or colourless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.no | vol. of sample(ml) | burette reading | | vol. of . hypo(ml) |
| initial | final |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

### **9.4.2.1 Calculation:-**

The amount of D.O in ppm =

= ppm

The B.O.D of the drinking water sample is = **D1-D2**

## **9.5 (b) Estimation of D.O for sewage water sample. (Before incubation) D1:-**

Reagent bottle is cleaned and filled with sewage water sample and it is immediately closed with a lid to prevent entering the atmospheric oxygen into the sample. Now add 5.0 mL of MnSO4 solution with the help of a syringe. In the same way add 5.0 mL of Sodium Azide until a brown precipitate is formed in the bottle. Same solutions are added again to form a complete precipitate in the bottle.

Now add con H2SO4 to the precipitate for complete dissolution. Now the dissolved solution is transferred in to a 250.0 mL conical flask and adds 1.0 mL of freshly prepared starch solution and kept this solution. The solution turns into dirty black now titrate this solution against hypo. The end point is the colour change from dirty black to blue or colourless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| s.no | vol. of sample(ml) | burette reading | | vol. of . hypo(ml) |
| initial | final |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

### **9.5.1 Calculation:-**

The amount of D.O in ppm = x1000

= ppm

## **9.6 Estimation of D.O for Sewage Water Sample. (After Incubation) D2:- (for 3 days at 270c)**

Reagent bottle is cleaned and filled with sewage water sample and it is immediately closed with a lid and covered with a parafin tape . Now put this bottle in the incubator **for 3 days at 270c**. After 3 days, add 5.0 mL of MnSO4 solution with the help of a syringe. In the same way add 5.0 mL of Sodium Azide until a brown precipitate is formed in the bottle. Same solutions are added again to form a complete precipitate in the bottle.

Now add con H2SO4 to the precipitate for complete dissolution. Now the dissolved solution is transferred in to a 250.0ml conical flask and add 1.0 mL of freshly prepared starch solution and kept this solution .the solution turns into dirty black now titrate this solution against hypo. The end point is the colour change from dirty black to blue or colourless.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| s.no | vol. of sample(ml) | burette reading | | vol. of . hypo(ml) |
| initial | final |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

## **9.6.1 Calculation:-**

The amount of D.O in ppm = x1000

= ppm

The B.O.D of the drinking water sample is = **D1-D2**

# **10 CHEMICAL OXYGEN DEMAND** for Drinking Water and Sewage Water Samples



## **10.1 Aim**: - To determine the chemical oxygen demand (C.O.D) for drinking water and sewage water sample.

## **10.2 Solutions required:-**

1. 0.25 N K2Cr2O7 for 500.0 mL.
2. 0.1N Fe (ii) for 500.0 mL.
3. Ferroien indicator (1.485g of 1, 10-phenonthralene +0.695 g of Feso4.7H2o in 100 mL water)
4. Con. H2SO4
5. AgSO4

**10.3 Theory:**

The organic matter present in sample gets oxidized completely by K2Cr2O7 in the presence of H2SO4 , AgSO4 , to produce CO2 and Water. The sample is refluxed with a known amount of K2Cr2O7 in the H2SO4 medium. The excess K2Cr2O7 is determined by titrating with standardized Fe (ii) using ferroin as an indicator. The K2Cr2O7 consumed by the sample is equivalent to the amount of oxygen required to oxidize the organic matter.

## **10.4 Standardization of Fe(ii) by using K2Cr2O7solution :-**

Pippet out10.0 mL 0.25N K2Cr2O7 of into a 250 mL conical flask and add 7.0 mL of con. HCl, 20.0 mL of water are added. To this add 2 to 3 drops of ferroin indicator. Now titrate this solution against Fe (ii). The end point is the color change from dark green 🡪 bluish green 🡪 red brown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. OF. K2CR2O7 | BURETTE READING | | VOL. OF FE(II)ML |
| INITIAL | FINAL |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

N1V1 = N2V2

N1= Normality of. K2Cr2O7, V1= Vol of K2Cr2O7 ,

N2= nor of Fe (ii) =? V2 = vol. of Fe (ii)

### (a) estimation of C.O.D for blank (distilled water):-

Take 50.0 mL of distilled water in to a 250.0 mL RBF. To this add 25 mL of 0.25 N K2Cr2O7, 1 g of AgSO4 and 7.0 mL of con. H2SO4 are added. Reflux the flask gently on water bath and the strongly at 150.0c for 2 hrs. Cool this solution at room temperature and measure the volume of the sample with measuring jar. Transfer this solution into a 250.0 mL CF and add 3 to 5 drops of ferroin indicator and titrate against std. Fe(ii) until the color changes from dark green to blue green to red brown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| s.no | vol. of. sample | burette reading | | vol. of Fe(ii)ml |
| Initial | final |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

## **10.5 Estimation of C.O.D for R.O. Water:-**

Take 50.0 mL of r.o water in to a 250.0 mL RBF. To this add 25 mL of 0.25N K2Cr2O7, 1 g of AgSO4 and 7.0 mL of con. H2SO4 are added. Reflux the flask gently on water bath and the strongly at 150.0c for 2 hrs. Cool this solution at room temperature and measure the volume of the sample with measuring jar. Transfer this solution into a 250.0 mL CF and add 3 to 5 drops of ferroin indicator and titrate against std. Fe(ii) until the color changes from dark green to blue green to red brown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.NO** | **VOL. OF. SAMPLE** | **BURETTE READING** | | **VOL. OF Fe(II)Ml** |
| **INITIAL** | **FINAL** |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

### **10.5.1 Calculation:**

Report:- the C.O.D of the drinking water is = --------mg/lit

## **10.6 (b) Estimation of C.O.D for Blank (Distilled Water):-**

Take 50.0 mL of distilled water in to a 250.0 mL RBF. To this add 25 mL of 0.25N K2Cr2O7, 1 g of AgSO4 and 7.0 mL of con. H2SO4 are added. Reflux the flask gently on water bath and the strongly at 150.0C for 2 hrs. Cool this solution at room temperature and measure the volume of the sample with measuring jar. transfer this solution into a 250.0 mL CF and add 3 to 5 drops of ferroin indicator and titrate against std. Fe(ii) until the color changes from dark green to blue green to red brown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. OF. SAMPLE | BURETTE READING | | VOL. OF Fe(II) |
| INITIAL | FINAL |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

## **10.7 Estimation of C.O.D for sewage water:-**

Take 50.0 mL of sewage water in to a 250.0 mL RBF. To this add 25 mL of 0.25 N K2Cr2O7, 1 g of AgSO4 and 7.0 mL of con. H2SO4 are added. Reflux the flask gently on water bath and the strongly at 150.0c for 2 hrs. Cool this solution at room temperature and measure the volume of the sample with measuring jar. Transfer this solution into a 250.0 mL CF and add 3 to 5 drops of ferroin indicator and titrate against std. Fe(ii) until the color changes from dark green to blue green to red brown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO | VOL. OF. SAMPLE | BURETTE READING | | VOL. OF Fe(II) |
| INITIAL | FINAL |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |

### **10.7.1 Calculation:-**

Report:- The C.O.D of the sewage water is = --------mg/lit

# **11 TOTAL SUSPENDED SOLIDS (TSS)**

Mass balances (an accounting of all mass of a pollutant in a defined system) are important concepts in environmental chemistry and geochemistry. Mass balance scan be conducted on any element or compound but are usually illustrated in the classroom using global mass balances of the water, nitrogen, sulfur, carbon, and phosphorus cycles. Examples of these can be found in Berner and Berner (1996). In this laboratory exercise, we will collect data and conduct a mass balance on inorganic and organic solids in a water sample. Due to the complexity of this experiment and time constraints, the class will be divided into three groups, with each group conducting a different experiment. Thus, everyone will have to keep careful records and share data with the rest of the class. But first we will answer two questions concerning suspended and dissolved solids in typical water samples:

## **11.1 Why are we concerned with total suspended solids (TSS)?**

* High concentrations of suspended solids may settle out onto a stream bedor lake bottom and cover aquatic organisms, eggs, or macro invertebrate larva. This coating can prevent sufficient oxygen transfer and result in the death of buried organisms.
* High concentrations of suspended solids decrease the effectiveness of drinking water disinfection agents by allowing microorganisms to ‘‘hide’’ from disinfectants within solid aggregates. This is one of the reasons that the TSS, or turbidity, is removed in drinking water treatment facilities.
* Many organic and inorganic pollutants sorb to soils so that the pollutant concentrations on the solids are high. Thus, sorbed pollutants (and solids)can be transported elsewhere in river and lake systems, resulting in the exposure of organisms to pollutants away from the point source.

## **11.2 Total Suspended Solids (TSS) and Suspended Volatilen Solids (SVS)**

### 11.2.1 Overview:

In this procedure you will be taking 100 mL of your sample and performing the most commonly used solids measurement, the total suspended solids. This requires you to filter a known volume of sample through a preheated and pretared glass-fiber filter. The difference in weights (final–initial) divided by the volume of sample will yield the TSS. The TSS measurement accounts for all solids that do not pass through the filter (typically, 0.45 to 1 mm in size), weighed after drying at 104\_C. When the filter is further dried to 550\_C, you will oxidize any organic matter present in the solids and can obtain suspended volatile solids measurement.

### 11.2.2 Step-by-Step Instructions

#### Preparing the Filters

1. Rinse three filters with 20 to 30 mL of deionized water to remove any solids that may remain from the manufacturing process. Place each filterin a separate, labeled aluminum weight pan, dry them in a 550\_Cmuffle furnace for 30 minutes, place them (filter and pan) in a Desiccator, and obtain a constant weight by repeating the oven and desiccation steps.

#### Obtaining the TSS Measurement

2. Filter 100 mL of sample through each filter.

3. Place each filter paper in the aluminum weight pan in the 104\_C oven for 1 hour. Cool the filter and pan in a desiccator and obtain a constant weight by repeating the drying and desiccation steps. This procedure will yield the TSS measurement (box 3 in Figure 20-1):

TSS (mg/L) = X1000

# 12. BACTERIOLOGICAL EXAMINATION OF WATER (METHOD-I)

**12.1 Aim:** To determine the Total microbial Count

## **12.2. Apparatus:**

* + 1. Petri Dish
    2. Incubator
    3. Measuring Cylinder, beaker, dropper etc.

## **12.3. Reagents:**

* 1. Appropriate culture medium (broth)
  2. Distilled water

## **12.4. Objectives**

* Carry out a presumptive test for the presence of coliform bacteria in a water sample
* Determine the most probable number (MPN) of bacteria in a positive presumptive sample
* Carry out a confirmed test to begin isolation of bacterial colonies
* Carry out a completed test using a Gram stain and morphology

## **12.5. Introduction**

We consider fresh-water streams, lakes, ground water or coastal ocean water to be polluted when some condition makes the water unsafe for human recreation or consumption. We usually think of two forms of pollution: toxic chemicals or pathogenic microorganisms. Probably the largest single source of potentially pathogenic microbes is animal feces (including human), which contains billions of bacteria per gram. Although most intestinal microbes are non-pathogenic, some cause enteric disease.

The organisms which cause typhoid fever (*Salmonella typhi*), cholera (*Vibrio cholera*), and bacterial dysentery (*Shigella flexneri*) are examples of enteric diseases caused by bacteria. In addition, some viral and protozoan pathogens are spread through water contaminated by feces. Testing for each organism separately would be extremely costly and time-consuming. Therefore, a simple rule is followed: if a water sample contains any microorganism common to animal intestines, it should not be consumed, because it may contain enteric pathogens. Water testing for microbiological safety rests on the ability of microbiologists to detect coliform bacteria. The word “coliform” refers to any bacterium that is like *Escherichia coli* in the following characteristics: 1) it is a small, gram-negative rod; 2) it does not contain spores; 3) it ferments lactose with the production of acid and gas; 4) it produces a green metallic sheen on EMB agar.

*E. coli*, which is found in large numbers in the feces of all animals, lives longer in water than most intestinal pathogens do. Therefore, if no *E. coli* are present, there should be no intestinal pathogens present in the water sample. For this reason, testing for coliform organisms is performed daily by municipal water departments and waste-water (sewage) treatment plants. It is regularly tested for in coastal sea water samples, as well as runoff water.

The bacterial examination of water has been standardized into three tests. The first, or presumptive test, is a screening test to sample water for the presence of coliform organisms. A series of lactose fermentation tubes are inoculated with the water sample. If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction. The presumptive test is also designed to estimate the concentration of coliform organisms, called the most probably number (MPN) in the water sample.

The confirmed test is a second screening procedure in which a gram-negative selective medium is used (like EMB). This also allows for the differentiation of coliform (producing a green metallic sheen) from non-coliform colonies. The completed test is performed on a typical, well-isolated colony to reaffirm gas production in lactose, and to determine the morphology and gram reaction of the isolate from a nutrient agar slant.

## **12.6. Procedure 1**

### **Presumptive Test and MPN**

Each group will need: 3 triple strength lactose tubes with brom-thymol blue 6 regular strength lactose tubes with brom-thymol blue Bio 3B Laboratory, Water Quality Page 2 of 3 Sterile water collection bottles for community sampling (or “doped” water samples) 10 ml and 1 ml sterile pipets

* Collect approximately 50 ml of water to be tested (from pools, streams, ocean, etc.) or you may use the “doped” samples provided in class. Record the source and date of community samples or the sample number of the “doped” sample to be tested.
* Vigorously shake the water sample to be tested by moving it 25 times through a 12- inch arch.
* Transfer 10 ml of the sample into each of the three, triple strength lactose tubes. Always use aseptic technique in the water inoculations and label the tubes with the amount of water sample tested.
* Transfer 1 ml of the sample into each of three regular strength lactose tubes. Using the same pipet, transfer 0.1 ml to each of the three remaining regular strength lactose tubes.
* Incubate all tubes at 37o C. until the next laboratory session.

## **12.7. Procedure 2**

### **Confirmed Test**

* 1. Examine the tubes from the presumptive test and determine if any has produced an acid/gas reaction. If so, this is a positive presumptive test. If no gas is present in any of the Durham tubes, this is a negative presumptive test.
  2. Determine the number of tubes positive for acid/gas in each of the three volume categories. Determine the MPN of your water sample by comparing these numbers to the MPN Determination chart accompanying this exercise. Complete the table for the presumptive test as shown below and enter it into your laboratory notebook.
  3. From any tube showing 10% gas production or more, streak one loopful of the broth onto an EMB plate using the isolation streaking technique. Incubate the plate at 37o C. until the next laboratory session.

## **12.8. Procedure 3**

### **Completed Test**

* + 1. Draw or describe your EMB plate in your notes. Carefully examine the plate, looking for well-isolated coliform colonies. Typically, E. coli colonies appear with a metallic green sheen on EMB. From one of these colonies, set up your completed test by inoculating a lactose fermentation tube and a NA slant. Incubate them at 37o C. until the next laboratory session.
    2. After this incubation, check the lactose tube for acid and gas production. If no gas is present, this is a negative completed test. Prepare a Gram stain from the NA slant. If the organism is a non-spore producing Gram negative rod and the lactose broth shows an acid/gas reaction, this is a positive completed test. Record your results in the table below in your laboratory notebook. Include a drawing of your Gram stain.

**12.9. Results**

### **Presumptive Test**

Record the source of the water sample tested and the number of tubes in each category that produced acid/gas reactions. Determine MPN and record in the following table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample Source | 10 ml tubes | 1.0 ml tubes | 0.1 ml tubes | MPN |
|  |  |  |  |  |

# **13. METHODS OF BACTERIOLOGICAL EXAMINATION OF WATER (METHODE-II)**

Basically there two methods of bacteriological analysis of water: (a) Multiple Tube or Most Probable Number (MPN) method, and (b) Membrane Filter (MF) method.

## **13.1. (a) Multiple Tube/ Most Probable Number (MPN) method:**

MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It’s based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculums placed into a set number of culture media tubes. Positive growth response after incubation may be indicated by such observations as gas production in fermentation tubes or visible turbidity in broth tubes, depending upon the type of media employed.

## **13.2. (b) Membrane Filter Method:**

In contrast to the multiple-tube (MT) method, the membrane filter (IVIF) method gives a direct count of total coliforms and fecal coliforms present in a given sample of water. The method is based on the filtration of a known volume of water through a membrane filter consisting of a cellulose compound with a uniform pore diameter of 0.45 μm; the bacteria are retained on the surface of the membrane filter. When the membrane containing the bacteria is incubated in a sterile container at an appropriate temperature with a selective differential culture medium, characteristic colonies of coliforms and fecal coliforms develop, which can be counted directly. This technique is popular with environmental engineers. This method is not suitable for turbid waters, but otherwise it has several advantages. Its particular advantages and limitations are as follows:

## **13.3. Test Procedure (For MF method):**

This section describes the general procedures, it should be noted that different types of filtration units and equipment are available in the market for performing the tests.

### 13.3.1. Determination of Total Coliforms (TC):

1. Connect the Erlenmeyer (side-arm) flask to the vacuum source (turned off) and place the porous support in position. if an electric pump is used, it is advisable to put a second flask between the Erlenmeyer and the vacuum source; this second flask acts as a water trap and thus protects the electric pump.
2. Open a Petri-dish and place a pad in it.
3. 'With a sterile pipette add 2 mL of selective broth (culture) medium to saturate the pad.
4. Assemble the filtration unit by placing sterile membrane filter on the porous support, using forceps sterilized earlier by flaming.
5. Place the upper container in position and secure it with the special clamps. The type of clamping to be used will depend on the type of equipment.
6. Pour tide volume of sample chosen as optimal, in accordance with the type of water, into the upper container. If the test sample is less than 10 mL, at least 20 ml of sterile dilution water should be added to the top container before filtration applying the vacuum.
7. After the sample has passed through the filter, disconnect the vacuum and rinse the container with 20-30 mL of sterile dilution water. Repeat the rinsing after all the water from the first rinse has passed through the filter.
8. Take the filtration unit apart and using the forceps, place the membrane filter in the Petri-dish on the pad with the grid side up. Make sure that no air bubbles are trapped between the pad and the filter.
9. Invert the Petri-dish for incubation.
10. Incubate at 35°C or 37°C for 18-24 hours with 100% humidity (to ensure this, place a piece of wet cotton wool in the incubator). If ointment containers or plastic dishes with tight-fitting lids are used, humidification is not necessary.

## **13.4. Bacterial Colony observation:**

Colonies of coliform bacteria are a medium red or dark red color, with a greenish gold or metallic surface sheen. This sheen may cover the entire colony or appear only in the centre of the colony. Colonies of other types should not be counted. The colonies can be counted with the aid of a lens. The number of total coliforms per 100 mL is then given by:

### 13.4.1. Determination of Fecal Coliforms (FC):

The procedure for fecal coliforms is similar to that used for determining total coliforms. Filter the sample as described, and place the membrane filler on the pad saturated with appropriate culture medium.

1. Place the dishes in an incubator at 44±0.5 °C for 24 hours at 100% humidity. Alternatively, tight-fitting or sealed Petri-dishes may be placed in water-proof plastic bags for incubation.
2. Submerge the bags in a water-bath maintained at 44±0.5°C for 24 hours. The plastic bags must be below the surface of the water throughout the incubation period. They can be held down by means of a suitable weight, e.g., a metal rack.

## **13.5. Bacterial Colony observation:**

Colonies of fecal coliform bacteria are blue in color. This color may cover the entire colony, or appear only in the center of the colony. Colonies of other types should not be counted. The colonies can be counted with the aid of a lens. The number of fecal coliforms per 100 ml is then given by:

**13.6.Calculation:**

Total coliform (CFU/ 100 mL) =

Fecal coliform (CFU/ 100 mL) =

**Table:**

|  |  |  |
| --- | --- | --- |
| **Observation No.** | **Total Coliform Unit per 100 ml** | **Fecal Coliform Unit per 100 ml** |
|  |  |  |
|  |  |  |

# **14. SILICA**

**14.1. Aim:** To Estimate the amount of Silica present in the given water sample

## **14.2. Reagents:**

1. HCl
2. H2SO4
3. HF

## **14.3. Apparatus:**

1. Platinum Dish
2. Hot Plate
3. Filter Paper

**14.4. Theory:** Silica is not a water pollutant but excess of silica is undesirable for several industrial uses, it forms difficult to remove silica and silicate scales in various equipment and moreover, it forms a pure silica deposit on high pressure steam turbine blades.

The Silica content of natural water is usually 1-30 mg/L but higher amount 100 mg/L are not unusual in some cases while brackish waters and brains may contain as high as 1000 mg/L of Silica. It is thus necessary to monitor the silica content of water body. The gravimetric method is useful for 20 mg/L or more of SiO2 and spectrophotometric method for 0.4 – 25 mg/L of SiO2.

## **14.5. Gravimetric Method:**

Silicates and dissolved SiO2 are decomposed by HCl giving silicic acids during evaporation and baking. Ignition completes dehydration of SiO2 which is weighed and then volatilized as SiF4 leaving impurities as nonvolatile residue. The residue is weighed and the difference gives SiO2 lost on volatilization.

Na2SiO3 ­(SiO2) + 2 HCl → 2 NaCl + H2SiO2

H2SiO3 ----------> SiO2

-H­2O

SiO2 + 4HF + 2H2SO4 -----------> SiF4 + 2H2O +2H2SO4

## **14.6. Procedure:**

1. Take a Clear sample (at least 10 mg SiO2) in a 200 mL Platinum dish, add 5 mL 6N HCl and evaporate repeatedly with addition of HCl to dryness on a water bath.
2. Bake the residue on a hot plate for half an hour
3. Leach with 5 mL 6N HCl, warm and add 50 mL hot distilled water. While hot, filter through on ashless filter paper. Wash the dish and residue with hot 0.2N HCl and then with small volume of distilled water till the filtrate is chloride free.
4. Evaporate the filtrate and washings from the above step to dryness in the original platinum dish and repeat steps 2 and 3 above.
5. Transfer the two filter papers and residues thus obtained to a covered and weighed platinum crucible, dry at 1100C and finally ignite at 12000C to constant weight.
6. Moisten the residue in the crucible with distilled water. Add 4 drops of 18N H2SO4 and then add 10 mL HF. Slowly evaporates to dryness over a hot plate in a hood. Ignite the crucible at 12000C to constant weight.
7. Record the weight of SiO2 as the difference in these two weights from the steps 5 and 6 above.

# **15. MLVSS (Mixed Liquor Volatile Suspended Solids)**

### **15.1 Aim:** Determine the TSS ,VSS, MLSS and MLVSS

## **15.2. Apparatus:**

* Dessicator
* Drying oven, for operation at 103 to 105°C
* Analytical balance, capable of weighing to 0.1 mg
* Magnetic stirrer with TFE stirring bar
* Wide-bore pipets
* Graduated cylinder
* Low-form beaker
* Glass-fiber filter disks with organic binder
* Filtration apparatus, which can be any one of the following:
  + Membrane filter funnel
  + Gooch crucible, 25 mL to 40 mL capacity, with Gooch crucible adapter
  + Filtration apparatus with reservoir and coarse fritted disk (40 to 60 um) as filter support
* Filter flasks, of sufficient capacity for sample size selected
* Vacuum pump
* Tubing
* Stop watch
* Aluminum weighing dishes
* Muffle furnace

**15.3. A. TSS (Total Suspended solid)  
15.3.1. Procedure of TSS (Total Suspended solid)**

#### Preparation of glass fiber filter disks:

* + - * 1. Insert the filter disk in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water.
        2. Continue suction to remove all traces of water, turn vacuum off, and discard washings.
        3. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish.
        4. Dry in an oven at 103 to 105°C for 1 h.
        5. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.
      1. Selection of sample size :

Choose sample volume to yield between 2.5 to 200mg dried residue.

* + - 1. Sample analysis:
         1. setup the filtration unit with the dried glass fiber filter disk. Apply the well stirred sample volume in the center of the filter. wash the filter with 10 ml of reagent grade water and continue suction for about 3 min after filtration is complete.
      2. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as a support.
      3. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

Calculation   
mg total suspended solids/L =(A-B) X 1000/sample volume, mL  
  
where:  
A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

## 15.4. B. VSS (volatile suspended solid)

### Procedure of VSS (volatile suspended solid)

All the above process is same accept ignition process. Here the the glass filter paper is ignited at 550 degree.

15.5. C. MLSS (Mixed Liquor Suspended Solids) & MLVSS(Mixed Liquor Volatile Suspended Solids)

Mixed liquor is a combination of sludge and water removed from the clarifier in the wastewater treatment process and reintroduced into an earlier phase of the treatment process. The mixed liquor contains microorganisms which digest the wastes in the raw water.

Mixed Liquor Suspended Solids (MLSS) is a test for the total suspended solids in a sample of mixed liquor. This test is essentially the same as the test you performed for TSS in the last lab, except for the use of mixed liquor as the water sample. In addition, the concentration of suspended solids found in the mixed liquor is typically much greater than that found in the raw or treated water. MLSS concentrations are often greater than 1,000 mg/L, but should not exceed 4,000 mg/L.  
MLVSS, or Mixed Liquor Volatile Suspended Solids, is a test for the amount of volatile suspended solids found in a sample of mixed liquor.Volatile solids are those solids which are burnt up when a sample is heated to 550°C.Most of the volatile solids in a sample of mixed liquor will consist of microorganisms and organic matter. As a result, the volatile solids concentration of mixed liquor is approximately equal to the amount of microorganisms in the water and can be used to determine whether there are enough microorganisms present to digest the sludge.

**Procedure:**

1) Collect a grab sample of mixed liquor.

1. Measure the total suspended solids (TSS). Record the sample volume and the combined sample and filter weight .At least 10% of all samples should be analyzed in duplicate.
2. Ignite the filter and the total suspended solids residue from step 1 in a muffle furnace at 550°C. An ignition time of 15 to 20 minutes is usually sufficient for 200 mg residue.
3. Let the filter cool partially in the air until most of the heat has dissipated. Then transfer the filter to a dessicator to cool the rest of the way to air temperature.
4. Weigh the filter and record the weight.
5. Repeat the cycle of igniting, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% or 0.5 mg, whichever is less.
6. Calculate the volatile solids in the sample, as follows:

mg total suspended solids/L = (A-B) X 1000/sample volume, mL

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

B = weight of filter, mg.

**16. ESTIMATION OF IRON (III) USING**

**THIOCYANATE COLORIMETRICALLY**

**AIM:**

To determine the amount of iron (III) present in the given solution colorimetrically.

**APPARATUS:**

Burettes – 4, volumetric flasks (50ml) – 16

**CHEMICALS REQUIRED**

Ferric alum (Ferric ammonium sulphate), hydrochloric acid, nitric acid, potassium thiocynate.

**PRINCIPLE:**

Ferric iron reacts with thiocyanate to give a series of intensely red – coloured compounds, which remains in true solution. Ferrous iron does not react. Depending upon the thiocyanate concentration, a series of complexes can be formulated as ****

At low thiocyanate concentration the predominant coloured species is 

at 0.1M thiocyanate concentration  and

at very high thiocyanate concentration it is largely 

In the colorimetric determination a large excess of thiocyanate should be used, since this increase the intensity and also the stability of the colour. Strong acids should be present to suppress hydrolysis. Sulphuric acid is not recommended because it has a tendency to form complexes with ferric ions.



**PREPARATION OF REAGENTS:**

**Preparation of standard iron (III) solution:**

About 0.864 g of ferric ammonium sulphate is accurately weighed and dissolved in distilled water. To it 10 ml of concentrated hydrochloric acid is added and the volume is made to one litre in a volumetric flask. One ml of this solution consists 0.1 mg of iron.

The solution can be standardized by reducing Fe(III) to Fe(II) using stannous chloride. Since it is very convenient to standardize iron (II) in presence of hydrochloric acid with dichromate solution.



To 25.0ml of hot ferric solution  containing 5 – 6 N hydrochloric acid is reduced by adding concentrated stannous chloride solution dropwise from a burette with stirring until the yellow colour of the solution has disappeared. The reduction is then completed by diluting the concentrated solution of stannous chloride with two volumes of dilute hydrochloric acid and adding the dilute solution dropwise, with agitation after each addition, until the liquid has a faint green colour, quite free from any tinge of yellow. The solution is then rapidly cooled under the tap to about with protection from the air and the slight excess of stannous chloride present is removed by adding 10ml of saturated solution of (5%) mercuric chloride rapidly in one portion and with through mixing. A silky white precipitate of mercurous chloride should be obtained.



The oxidizing agent has no appreciable effect upon the small amount of mercurous chloride in suspension. Thus obtained iron (II) solution by reduction is standardized using standard potassium dichromate solution.

**Preparation of potassium thiocyanate solution (20%):**

About 20 g of potassium thiocyanate is dissolved in 100 ml of water, the solution is 2M.

**Preparation of 4N nitric acid:**

125 ml of concentrated nitric acid is diluted to 500 ml using distilled water.

**PROCEDURE:**

A standard series of different concentrations of iron (III) solutions are required for the preparation of calibration curve. About 10 to 12, 50ml standard volumetric flask numbered in a serial order are taken. To each flask 5ml of potassium thiocyanate (2M) and 4ml of nitric acid (4N) are added. Then about 25ml of distilled water is added to all the flasks such that the total volume in each flask ranges from 30 to 35ml. Then iron (III) solution is added to each one of the flasks starting from 0.5ml. Then immediately the contents are made upto the mark, thoroughly mixed and the optical density is measured at 480mm against the reagent blank. A reagent blank is one in which both 4ml of nitric acid (4N), 5ml of potassium thiocyanate (2M) are taken and the flask is made upto the mark with distilled water. The successive measurements are done with incremental additions of different concentrations of iron (III) solution to the rest of the flasks. The optical densities are found against the blank solution for each concentration at  (480mm). This must be done as soon as after preparation, since there will be variations in colour intensities owing to the formation of different complexes of iron – thiocyanate based on the ratios of iron and thiocyanate at each concentration of iron taken for the colour development.

A calibration curve is constructed taking amount of iron (III) on x axis and corresponding optical densities on y-axis and the curve is checked whether the Beer’s law is obeyed or not. The unknown solutions are similarlytaken and the colour intensity (optical density is measured) from which the concentration of iron (III) present in unknown solutions are computed.

**Precautions:**

1. Sulphuric acid should not be used to suppress the hydrolysis of ferric ions.
2. The optical densities must be taken as soon as after preparation (i.e., after colour development)
3. There should not be any time lag after the addition of iron (III) solution in colour development process.

**Report:** The amount of iron (III) present in the given 100ml solution is \_\_\_\_\_\_mg.

**OBSERVATIONS AND CALCULATIONS:**

**For drawing Beers law plot:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No.** | **Volume of**  **KSCN ml** | **Volume of**  **4N HNO3(ml)** | **Volume of**  **Fe(III) solution** | **Amount of iron**  **(mg)** | **Optical**  **Density** |
| 1 | 5.0 | 4.0 | 0.5 | - | - |
| 2 | 5.0 | 4.0 | 1.0 | - | - |
| 3 | 5.0 | 4.0 | 1.5 | - | - |
| 4 | 5.0 | 4.0 | 2.0 | - | - |
| 5 | 5.0 | 4.0 | 2.5 | - | - |
| 6 | 5.0 | 4.0 | 3.0 | - | - |
| 7 | 5.0 | 4.0 | 3.5 | - | - |
| 8 | 5.0 | 4.0 | 4.0 | - | - |
| 9 | 5.0 | 4.0 | 4.5 | - | - |
| 10 | 5.0 | 4.0 | 5.0 | - | - |

**For the determination of amounts from Beers law plot:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No.** | **Volume of**  **KSCN ml** | **Volume of**  **4N HNO3(ml)** | **Volume of**  **Fe(III) solution** | **Amount of iron**  **(mg)** | **Optical**  **Density** |
| 11 | 5.0 | 4.0 |  | - | **OD** |
| 12 | 5.0 | 4.0 |  | - | **OD** |
| 13 | 5.0 | 4.0 |  | - | **OD** |
| 14 | 5.0 | 4.0 |  | - | **OD** |

w4

w3

w2

w1

**O.Dy2**

**O.Dy1**

**O.Dx2**

**O.Dx1**

Amount of iron

**From graph:**

  ml of the given solution contains  mg of iron (III)

Then 100 ml of the given solution contains of iron (III)

  ml of the given solution contains mg of iron (III)

Then 100 ml of the given solution contains  of iron (III)

 ml of the given solution contains mg of iron (III)

Then 100 ml of the given solution contains of iron (III)

 ml of the given solution contains mg of iron (III)

Then 100 ml of the given solution contains of iron (III)

**Report:**

The amount of the iron (III) present in the given 100ml of unknown solution is as follows:

 = Amount of iron (III) present in unknown solution A (original)=\_\_\_\_\_mg

 =Amount of iron (III) present in unknown solution A(duplicate)=\_\_\_\_\_\_mg

 = Amount of iron (III) present in unknown solution A (original)=\_\_\_\_\_mg

 =Amount of iron (III) present in unknown solution A(duplicate)=\_\_\_\_\_\_mg

**17. ESTIMATION OF MANGANESE (II) BY**

**PERIODATE OXIDATION COLORIMETRICALLY**

**AIM:**

To determine the amount of manganese (II) present in the given solution colorimetrically.

**APPARATUS:**

Burettes – 2, graduated pipette (5ml) – 1, volumetric flasks (50ml) – 12, volumetric flasks (500ml), volumetric flasks (250ml), volumetric flasks (100ml)

**CHEMICALS REQUIRED:**

Potassium permanganate, oxalic acid, sodium sulphite, potassium periodate, phosphoric acid (manganese free), sulphuric acid.

**PRINCIPLE:**

Manganese in small quantities can be estimated by oxidation to permanganic acid. The oxidizing agent is potassium periodate. In hot acid solution periodate oxidizes manganese ion quantitatively to permanganic acid.



Permanganic acid is pink coloured and the colour produced is proportional to the present.

**PREPARATION OF REAGENTS:**

**Preparation of  solution:**

A standard is prepared by dissolving 0.8 g of in 500 ml water and its normality is found by titrating with standard 0.05N oxalic acid

solution. 100ml of this solution is quantitatively transferred into 1000ml beaker and 20ml of 10N is added to maintain 2N acidity. To this solution saturated sodium sulphite solution is added until the contents are colourless and boiled to evaporate excess sodium sulphite until no fumes are evolved. The solution is colourless and it is quantitatively

transferred to 250ml volumetric flask and made up to the mark. This is the stock solution.

**Working solution:**

50ml of stock solution is diluted to 100ml in a volumetric flask using distilled water.

**Note: Alternatively suitable dilutions are advised depending upon the colour intensity that is measured by trial and error method**

**PROCEDURE:**

**Standardization of  solution:**

20.0 ml of standard oxalic acid solution is transferred to a 250 ml conical flask and it is titrated against solution by maintaining 2N acidity at temperature. The end point is colourless to pale pink.

**Determination of for maximum absorbance:**

**Note: This is given only for training the student in getting absorption spectrum and hence locating the maximum absorbance for the coloured system.**

1 ml of 1N , 5ml of orthophosphoric acid (manganese free), 2mg of potassium periodate are added to ‘x’ (where x is any volume between 1 to 10ml) of  solution and made about 40ml in a 50ml volumetric flask in order to keep room for expansion during heating. The solution is heated by keeping the flask in a water bath for 10 minutes and suddenly cooled. The solution is made up to the mark with distilled water. The absorbances are noted at various wavelengths between 450-570mm and graph is drawn between wavelength and optical density. The graph shows maximum absorbance  at 545mm.

**Estimation of Mn(II) present in the given solution:**

1 to 10ml of  solution is taken in 50ml volumetric flasks. Then to each flask 1ml of sulphuric acid (1N) and 5ml of phosphoric acid (manganese free) are added. 2mg of potassium periodate is added to each of them. A blank solution is also made in a similar manner without  solution. The contents of the flasks are made approximately say to 40ml.

They are heated in a water bath for 10min. and suddenly cooled and made up to the mark. The wave length is set at 545mm and optical densities are measured for different concentrations to draw a Beer’s law plot.

A graph is drawn by taking amount of  present on X-axis and optical density on Y-axis. A straight line is obtained. Same procedure is adopted for the given unknown samples also and the amount of  in unknown samples is computed from the graph.

**PRECAUTIONS:**

1. The amount of manganese should not exceed 2mg per 100ml, otherwise the colour will be too dark and hence will be out of range of determination.
2. Before periodate oxidation, excess sodium sulphite solution should be removed by expelling SO2 completely.
3. Phosphoric acid should be added to the test solution, which prevent the precipitation of ferric iodate and also decolourises ferric iron by complex formation.

**Report:** The amount of manganese (II) present in the given 100 ml solution is \_\_\_\_\_\_mg.

**OBSERVATIONS AND CALCULATIONS:**



**Standardisation of KMnO4 solution:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Volume of oxalic acid (v1 ml)** | **Burette readings** | | **Volume of KMnO4 solution (v2 ml)** |
| **Initial** | **Final** |  |
| 1. | 20.0 |  |  |  |
| 2. | 20.0 |  |  |  |
| 3. | 20.0 |  |  |  |

We know that 



Amount of manganese present in the 500ml solution = w1





Since 250ml of stock solution is prepared from 100ml of permanganate solution.

Amount of manganese present in the 250ml of stock solution = Amount of manganese present in



Since 100ml of working solution is prepared by diluting 50ml of stock solution.

Amount of manganese present in the 100ml working solution = Amount of manganese present.



Therefore, amount of manganese (II) present in 1 ml of working solution



**Determination of :**

|  |  |
| --- | --- |
| **Wavelength** | **O.D.** |
| 400 |  |
| 410 |  |
| 420 | B |
| 430 |  |
| 440 |  |
| 450 | C  Optical Density |
| 460 |  |
| 470 | A |
| 480 |  |
| 490 | D |
| 500 |  |
| 510 |  |
| 520 |  |
| 530 | 400 450 500 550 |
| 540 |  |
| 550 |  |



**Determination of manganese (II):**

**For drawing Beers law plot:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |
| 1 | 1.0 | 5.0 | 2.0 | 1.0 |  |  |
| 2 | 1.0 | 5.0 | 2.0 | 2.0 |  |  |
| 3 | 1.0 | 5.0 | 2.0 | 3.0 |  |  |
| 4 | 1.0 | 5.0 | 2.0 | 4.0 |  |  |
| 5 | 1.0 | 5.0 | 2.0 | 5.0 |  |  |
| 6 | 1.0 | 5.0 | 2.0 | 6.0 |  |  |
| 7 | 1.0 | 5.0 | 2.0 | 7.0 |  |  |
| 8 | 1.0 | 5.0 | 2.0 | 8.0 |  |  |
| 9 | 1.0 | 5.0 | 2.0 | 9.0 |  |  |
| 10 | 1.0 | 5.0 | 2.0 | 10.0 |  |  |

**For the determination of amounts from Beers law plot:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No.** | **Volume of**  **(ml)** | **Volume of**  **(ml)** | **Wt. of period date**  **(mg)** | **Volume of working Mn(II) solution (ml)** | **Amount of Manganese(II)**  **(mg)** | **Optical Density** |
| 11 | 1.0 | 5.0 | 2.0 |  | - |  |
| 12 | 1.0 | 5.0 | 2.0 |  | - |  |
| 13 | 1.0 | 5.0 | 2.0 |  | - |  |
| 14 | 1.0 | 5.0 | 2.0 |  | - |  |

w4

w3

w2

w1

**O.Dy2**

**O.Dy1**

**O.Dx2**

**O.Dx1**

Amount of manganese(II)

**From graph:**

  ml of the given solution contains  mg of manganese (II)

Then 100 ml of the given solution contains of manganese (II)

  ml of the given solution contains mg of manganese (II)

Then 100 ml of the given solution contains  of manganese (II)

 ml of the given solution contains mg of manganese (II)

Then 100 ml of the given solution contains of manganese (II)

 ml of the given solution contains mg of manganese (II)

Then 100 ml of the given solution contains of manganese (II)

**Report:**

The amount of the iron (III) present in the given 100ml of unknown solution is as follows:

 = Amount of manganese (II) present in unknown solution A (original)=\_\_\_\_\_mg

 =Amount of manganese (II) present in unknown solution A(duplicate)=\_\_\_\_\_\_mg

 = Amount of manganese (II) present in unknown solution A (original)=\_\_\_\_\_mg

 =Amount of manganese (II) present in unknown solution A(duplicate)=\_\_\_\_\_\_mg

**18. CONDUCTOMETRIC DETERMINATION OF**

**STRONG ACID AND WEAK ACID IN A**

**MIXTURE WITH STRONG BASE**

**AIM:**

To determine the strength of a strong acid (hydrochloric acid) and a weak acid (acetic acid) in the given mixture.

**APPARATUS:**

Burette, volumetric flasks (250ml), beaker (250ml)

**CHEMICALS REQUIRED:**

Sodium hydroxide, oxalic acid, hydrochloric acid, acetic acid, phenolphthalein indicator.

**THEORY:**

This type of titration is just a combination of two separate titrations viz.,  against and against  By adding alkali to the mixture, the conductivity of the solution decreases due to the replacement of ions from the strong acid. It then increases as the weak acid is converted into salt and finally rises more steeply as excess of alkali is added.

A curve is plotted with conductivity as ordinate (y-axis) against volume of alkali added as abscissa (x-axis). It is observed that there is rounding off at both the end points. Usually extrapolation of the straight lines of the three branches would lead to a definite location of the end points. It must be noted that the first end point will be that of hydrochloric acid (strong) while the second is for that of acetic acid (weak).

**PREPARATION OF REAGENTS:**

**Preparation of double distilled water (conductivity water):**

Ordinary distilled water posses large conductance due to materials dissolved from the container and also due to the presence of carbondioxide and ammonia dissolved from the air. So it is quite unsuitable for conductivity measurements. Hence, conductivity water must be used. It can be prepared by the distillation of about 2 litres of distilled water in which about 8 to 10 crystals of potassium permanganate and 2 to 3 crystals of sodium hydroxide are added. The contents are boiled for about 10 to 15 minutes and the distillation is carried out. Discarding the head and tail portions the water vapour (steam) coming out of the outlet of flask are condensed and collected into ground joint bottles. It should not be preserved more than a week and hence a freshly prepared conductivity water has to be used.

**Preparation of standard exalic acid solution (0.1N):**

About 3.15 g of oxalic acid dehydrate is weighed accurately and dissolved in small amount of distilled water and made up to the mark in a 250ml volumetric flask.

**Preparation of sodium hydroxide (0.1N):**

About one gram of sodium hydroxide is dissolved in small amount of water and diluted to 250ml and it is standardized against standard oxalic acid solution.

**PROCEDURE:**

**Standardisation of sodium hydroxide solution:**

10.0ml of oxalic acid is pipette out into a conical flask and it is diluted to 30ml with distilled water. To the solution two or three drops of phenolphthalein indicator is added and then titrated against sodium hydroxide taken in a burette. The end point is colourless to pale pink. The experiment is repeated until concurrent readings are obtained

**Determination of strength of hydrochloric acid and acetic acid:**

10.0ml of the given mixture of hydrochloric acid and acetic acid is pipette out into a 400ml beaker and about 100ml of distilled water is added. The electrode of the cell is dipped in the solution of mixture. The beaker is placed in a water bath to maintain constant temperature.

The conductivity of the solution is noted before adding the alkali. Then standardized sodium hydroxide is added from a burette with 1ml increment and the conductivities are noted while shaking thoroughly the contents of the beaker during the addition. Near the equivalence sodium hydroxide is added drop wise. To get a neat curve the titration is continued with a few more increments.

The values of observed conductivity are plotted as ordinate against volume of sodium hydroxide added as abscissa. The concentrations of hydrochloric acid and acetic acid are determined using the end points from the graph.

**Precautions:**

1. After each addition of the titrant from the burette, the solution should be thoroughly stirred for about a minute and then the reading should be taken.
2. Just before and after the end point, the addition of titrant should be in as small fractions as possible.

**Report:** The strength of each acid in the give mixture is.

1. Hydrochloric acid =\_\_\_\_\_\_\_\_\_\_g/lit.
2. Acetic acid =\_\_\_\_\_\_\_\_\_\_g/lit.

**Observations and calculations:**



**Standardisation of sodium hydroxide solution:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Volume of oxalic acid** | **Burette readings** | | **Volume of solution** |
| **Initial** | **Final** |
| 1. | 20.0 |  |  |  |
| 2. | 20.0 |  |  |  |
| 3. | 20.0 |  |  |  |

**Determination of strength of hydrochloric acid and acetic acid:**

V3 = Volume of mixture of hydrochloric acid and acetic acid solution = 10.0ml

|  |  |
| --- | --- |
| **PILOT OF TITRATION** | |
| **Volume of**  **NaOH (ml)** | **Conductivity** |
|  |  |

|  |  |
| --- | --- |
| **REGULAR TITRATION** | |
| **Volume of**  **NaOH (ml)** | **Conductivity** |
|  |  |

conductance

(ohm-1)

Volume of sodium hydroxide

**Determination of amount of hydrochloride acid**



Strength of hydrochloric acid = Normality of hydrochloric acid solution x Eq.Wt. of HCl



**Determination of amount of acetic acid:**



Strength of acetic acid = Normality of acetic acid solution x Eq.Wt. of acetic acid.



**Note: The mixture of HCl and CH3COOH shall be prepared in such a way that the first equivalence point will lie near about the addition of 5ml, while the second equivalence point will be near about addition of another 5ml (10ml) of sodium hydroxide.**

**19. pH METRIC DETERMINATION OF**

**AN ORGANIC ACID**

**AIM:**

To determine the strength of an organic acid (acetic acid) using pH meter.

**APPARATUS:**

Burette, volumetric flasks (250ml), beaker (250ml)

**CHEMICALS REQUIRED:**

Sodium hydroxide, oxalic acid, acetic acid, phenolphthalein indicator.

**THEORY:**

When an alkali is added to an acid solution, the pH of the solution increases slowly, but at the vicinity of the equivalence point, the rate of change of pH of the solution is very rapid. From the sharp break in the curve, we can find the equivalence point, from which the strength can be calculated by usual mathematical relation .

**Preparation of standard oxalic acid solution (0.1N):**

About 1.5759 g of oxalic acid dehydrate  is weighed accurately and dissolved in small amount of distilled water and made up to the mark in a 250ml volumetric flask.

**Preparation of sodium hydroxide (0.1N):**

About one gram of sodium hydroxide is dissolved in small amount of water and diluted to 250ml and it is standardized against standard oxalic acid solution.

**PROCEDURE:**

**Standardisation of sodium hydroxide solution:**

10.0ml of oxalic acid is pipette out into a conical flask and it is diluted to 30ml with distilled water. To the solution two or three drops of phenolphthalein indicator are added and then titrated against sodium hydroxide taken in a burette. The end point is colourless to pale pink. The experiment is repeated until concurrent readings are obtained.

**Determination of strength of acetic acid:**

The pH meter is standardized first against a buffer of known pH. The glass electrode and reference electrodes are washed with distilled water.

10.0ml of the given acetic acid solution is pipette out into a 100ml beaker and about 40ml of distilled water is added so that the tips of glass electrode as well as the reference electrode are completely immersed in solution. The pH of the solution is noted before adding the alkali. The standardized sodium hydroxide is added from a burette with 1 ml increment and the readings (pH) are noted while shaking thoroughly the contents of the beaker during the addition. Near the equivalence point sodium hydroxide is added drop wise. To get a neat curve titration is continued with a few more increments.

A curve is plotted with pH values as ordinate and the volume of alkali added as abscissa. The concentrations of acetic acid is determined using the end point from the graph.

**Precautions:**

1. After the each addition of the titrant from the burette, the solution should be thoroughly stirred for about a minute and then the reading should be taken.
2. Just before and after the end point, the addition of titrant should be of as small fractions as possible.
3. The temperature control knob of the pH meter should be adjusted to the room temperature.
4. The pH meter should be first standardized by taking a buffer solution of known pH.

**Report:** The strength of acetic acid is :­­\_\_\_\_\_\_\_\_\_\_g/lit

**OBSERVATIONS AND CALCULATIONS:**



**Standardisation of sodium hydroxide solution:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Volume of oxalic acid**  **(v1 ml)** | **Burette readings** | | **Volume of NaOH solution**  **(v2 ml)** |
| **Initial** | **Final** |
| 1. | 20.0 |  |  |  |
| 2. | 20.0 |  |  |  |
| 3. | 20.0 |  |  |  |



**Determination of strength of hydrochloric acid and acetic acid:**

|  |  |
| --- | --- |
| **REGULAR TITRATION** | |
| **Volume of**  **NaOH (ml)** | **pH** |
|  |  |

V3 = Volume of mixture of hydrochloric acid and acetic acid solution = 10.0ml

|  |  |
| --- | --- |
| **PILOT OF TITRATION** | |
| **Volume of**  **NaOH (ml)** | **pH** |
|  |  |



pH

Volume of sodium hydroxide

Strength of acetic acid = Normality of acetic acid solution x Eq.Wt. of acetic acid



**20. ANALYSIS OF COAL**

* Coal is a highly carbonaceous matter which is produced due to the change of vegetable matter, eg., plants under certain favourable conditions.
* It is mainly compared of C, H, N and O besides non-combustible inorganic matter.
* The assessment of quality of coal is made by the following two types of analysis:  
  a) Proximate Analysis  
  b) Ultimate Analysis

1. **Proximate Analysis:** It is an important analysis, which records moisture, volatile carbanaceous matter, ash and fixed carbon as percentage of the original weight of the coal sample.

* This analysis can be done by following methods:

1. **Moisture:** About 1 to 2 gms of dry air powder coal (60 mesh) is taken in a shallow silica crucible (the silica crucible is made constant and then accurately weighed coal is taken in it by themethod of difference) and heated in an electric over for 1 hour at a temperature of 1070C ± 20C for one hour.

* Then the crucible is taken out, cooled in a dessicator and weighed. The amount of weight loss is reported as moisture (on percentage basis)



1. **Volatile Carbonaceous Matter:**

* The dried sample of coal left in the crucible from (1) above is then covered with a lid and kept in a multiple furnace at 925 ± 250C for 7 minutes.
* After 7 minutes, the crucible is taken out and coded first in air and then inside the dessicator and weighed again. Loss weight is reported as volatile matter on percentage basis.



1. **Ash:** The determination of ash is done in a platinum crucible with lid (made constant) 1 or 2 gms finely powdered coal is at first heated carefully on a bunsen burner.

* The lid is then removed and strongly heated to burn any tarry matter adhering to it. The lid is kept in the dessicator.
* The heating of the crucible with coal is continued at a temperature of nearly 8000C in a muffled furnace, until the coal is completely burnt leaving behind the ash.
* The crucible is then cooled in the dessicator and weighed. Heating coding and weighing are repeated till constant weight is obtained.



1. **Fixed Carbon:**

* The residue left in the course of determination of volatile carbonaceous matter is the fixed carbon and ash. This fixed carbon is left as a result of destructive distillation of coal.
* Knowing the weight of ash, it is deducted from the weight of the residue. Then the percentage of fixed carbon can be reported as:



**21. ANALYSIS OF IRON ORE**

**Determination of Moisture:**

5 – 10 gm of the sample is weighed in a 7.5 cm watch glass and heated in air over at 1050C for about 2 hours or until the weight is constant. It is then cooled and weighed. The loss in weight divided by the weight of the sample and multiplied by 100 gives the percentage of moisture in the ore.

**Determination of loss of Ignition:**

Weigh 1.0 gm ore sample in a platinum or porcelain crucible. Ignite first at about 5000C. For 5 minutes and then at 9500C for 30 minutes. Cool and weigh loss in weight multiplied by 100 gives the percentage loss on Ignition.

**Determination of combined water:**

Put 1.0 gm weighed sample in a previously ignited porcelain boat place the boat with its content in a pyrex glass tube (½” dia × 12” long) and insert stoppers. At the inlet end of the tube connect a suitable desiccant. Such as H2SO4, CaCl2, or an hydrol. The exit end carries a weighed calcium chloride tube connected to a gentle suction commence suction and heat the boat from outside by a Bunsen burner to a temperature of about 3000C. For about 15 minutes weigh the CaCl2 tube again. The increase in weight multiplied by 100 gives the percentage of combined water.

**Determination of Total Iron:**

**Potassium dichromate method:**

Oxide ores brought into solution by HCl and sulphide ores by aqua regra. Both are evaporated to fumes with H2SO4 and re dissolved in conc Hcl. The Ferric chloride formed is converted to Ferrous condition by adding a solution of stannous chloride. In the boiling hot solution of the ore. The excess of stannous chloride is neutralized by adding a solution of mercuric chloride to the cold ferrous solution. This is then titrated with a standard potassium dichromate solution, using potassium ferricyanide as an external indicator. This method has a distinct advantage over the zinc reduction method in that it takes only a minute or two for the reduction to be completed.

**Solutions required:**

1. Standard 5/56N potassium dichromate solution. Dissolve 439 gm K2Cr2O7 in water and make the volume to 1 litre. Solution of any other known strength may be used, but because 1 ml of 5/56N K2Cr2O7 solution corresponds to 0.005 gm iron, the number of ml of this solution used will directly give the percentage of iron on a 0.5 gm sample of the ore.
2. **Stannous chloride solution:** 50 gm stannous chloride is dissolved in 200 ml ConC Hcl and then made up to 1 litre by adding water. The solution is preserved by adding a few pieces of granulated tin, so that any SnCl4 formed by atmospheric oxidation is again reduced to SnCl­2.
3. A saturated solution of mercuric chloride in water at room temperature.
4. A very dilute solution of potassium ferricyanide in water.

**Procedure:**

Weigh accurately 0.5 gm of the ore sample in a 250 ml conical flask and proceed exactly as in the determination or iron by kmno4 till you get a 10 ml dilute solution of the ore. Heat this solution to boiling and add to it stannous chloride solution drop by drop, all the time shaking the conical flask till the red colour of ferric iron is completely discharged. Add a few drops more of SnCl2 solution and cool quickly to room temperature by putting the flask under running tap water. The addition of stannous chloride should be made by a dropping bottle to avoid any large excess in the solution because its presence will consume more K2Cr2O7 solution, during titration and would thus register a higher percentage of iron. A slight excess of SnCl2 solution is added to ensure complete reduction of ferric iron to ferrous condition. Quick cooling of the solution after the addition of SnCl2 is necessary to prevent re-oxidation of the reduced solution by atmospheric oxygen.

Add about 10 ml of mercuric chloride (HgCl2) solution to the above cold reduced solution and dilute to about 200 ml. The addition of HgCl­2 is made to take care of the excess SnCl2. Also this addition is done in the cold reduced solution, because in the hot condition the reaction.



May take place, precipitating mercury in a very finally divided state. This mercury will be oxidized by K2Cr2O7 and will there or record a higher percentage of iron.

Now run standard K2Cr2O7 solution in the cold reduced solution through a burette. First liberally and then in small amounts stirring all the time with a glass rod. Take out a drop or the solution from the beaker each time you add K2Cr2O7 and mix it with a drop of freshly prepared potassium ferrycyanide solution on a spot plate. If a blue colour develops it is an indication that all ferrous iron is not oxidized to Fe(ic) condition. Continue adding dichromate solution in fractions of 1 to 2 ml till no blue colour develops with a drop or K3Fe(CN)6 solution. This indicate the end portion.

An internal indicator for the above titration has been developed. It consists of a solution of 1 gm. DPA in 100 ml ConC H2SO­4. 2 – 3 drops of this indicator is sufficient for one titration. This indicator gives an intense violet-blue colour when the oxidation of ferrous to ferric iron is completed. The sharpness of the end point is improved by the addition of 20 ml solution of 150 ml H2SO4 (1.84 sp.gr), 150 ml ortho phosphoric acid (1.7 sp.gr.) and 700 ml water.

The important reactions which take place during the determination of iron by the dichromate method are as follows.



**Determination of Silica:**

Mix 0.5 gm accurately weighed sample of iron ore with 3-4 gm of fusion mixture. Heat the mixed ore in a platinum crucible covered with a lid for 5 minute at 5000C and then for 15 minutes at 9500C. Cool the crucible and extract the melt in a porcelain basin, with the minimum amount of 1:1 hot Hcl wash the lid and crucible with cold distill water and if necessary boil with 1.16 sp.gr. Hcl on a hot plate. Transfer this to the original extract and wash the crucible again with water. Add 20 ml more of the 1.16 sp.gr Hcl in the basin and evaporate to dryness on a hot plate, preventing any loss by spattering. Spattering cool and moisture the dry cake with a couple of ml of Hcl (sp.gr. 1.16). Take to dryness again and bake strongly until the cake is almost black in colour. Cool and dissolve all iron salts in

30 ml 1.16 sp.gr Hcl. Dilute with equal volume of hot distilled water and boil. Filter through a pulp pad previously washed with dil Hcl collect the filtrate in a 500 ml conical flask. Wash the basin properly with 1:1 hot Hcl and hot distilled water several times, pouring everything on the pulp pad and collecting the filtrate into the same flask. Dry the pad and transfer the precipitate along with the pad to silica crucible. Ignite, coal and weigh. The weight residue in milligrams multiplied by 200 will give the percentage of silica in the ore sample.

To obtain more accurate result, silica should be determined by hydro fluorisation. In this case, the pad and the precipitate are ignited in a platinum crucible. The residue after ignition is weighed, moistered with a few drops of H2SO4 and added with 1 – 2 ml of HF. Evaporate to dryness and ignite again. Silica volatilizes as S­1F4. Weigh again the loss in weight gives the amount of silica on a 0.5 gm sample.

Before adding HF to the residue the addition of H2SO4 is necessary, else other oxides will also be converted to fluorides and will be voltalised.

The filtrate after the removal of S1O2 is used to determine Al2O3, Cao, mgO and TiO2 if present.

The filtrate is oxidized with 1 – 2 ml 1.42 sp.gr HNO3 and boiled to reduce the bulk to about 100 ml. Cool the solution and add 2 ml of bromine. Add 5 – 10 gm NH4Cl and sufficient quantity of ammonia to make it just ammonia Cal. Boil for 2 – 3 minutes to the except NH3. Filter through a filter paper, collecting the filtrate in a 750 ml conical flask wash the precipitate thoroughly with hot water and transfer to original conical flask. Dissolve the precipitate in 6 ml 1.16 sp.gr Hcl. Repeat the bromine and ammonia separation as above and filter through the original filter paper. Again wash the precipitation thoroughly with hot water, collecting all the filtrate in the 750 ml flask. Reserve the filtrate (A) for the determination of CaO and MgO. The precipitate (B) is used for determination of Al2O3 and any TiO2 present.

**Determination of alumina:**

Dissolve the hydroxide precipitate (B) in 1.16 sp.gr Hcl. To this solution add 15 ml of a cold saturated solution of a sodium phosphate and then add slowly 0.82 sp.gr NH4OH till a faint precipitate is formed. Dissolve this precipitate with a minimum excess of 1.16 sp.gr Hcl. Add 12 ml of glacial acetic acid and 10 gm of

sodium thiosulphate crystals. These are added to keep to keep iron in the solution in the ferrous state. Dilute with boiling distilled water and then boil for 10 – 15 minutes. Add 5 ml of ammonium acetate and boil for 5 – 10 minutes more. Filter through a filter paper and wash well. Dry the precipitate on a hot plate in a silica crucible and ignite gently at first and then at 9000C for 5 minutes. Cool and weighed the residue as AlPO4. The weight of the residue in milligrams multiplied by 83.6 will give the percentage of Al2O3 on a 0.5 gm ore sample.

**Determination of lime:**

**Rapid determination of lime:**

Sometimes it is necessary to determine Cao rapidly to control the blast furnace. For this, weigh 0.2 gm or the ore sample directly in a porcelain crucible and ignite at about 5000C for 2 minutes. Cool and transfer to a 400 ml beaker. Add exactly 10 ml 1.16 sp.gr Hcl from a burette and digest for 2 minutes. Then add 0.2 gm NaF. Digest until the residue is white. Dilute to 50 ml hot water. Add 25% NH4OH solution till a precipitate just forms. Add dil Hcl slowly to destroy this precipitate. Dilute to 50 ml with hot distilled water add 2 gm ammonium oxalate crystals. Shake until all the iron is reduced and calcium is precipitated as oxalate. Dilute to 100 ml with boiling water and boil further for 2 minutes. Allow to stand for 10 minutes filter and wash. Dissolve the precipitate in H2SO4 and titrate with a standard KmnO4 Sal’n as described in the previous case.

**Determination of Magnesia:**

For blast furnace control it may sometimes be necessary to have Mgo analysis quickly. In that case, determination may be done on a fresh sample of the ore when 0.5 gm of it is weighed in a 1” porcelain crucible. Ignite it gently for about 2 minutes at the mouth of a muffle furnace. Coal and transfer to a 250 ml beaker. Add 15 ml of an acid mixture (80 ml Hcl + 40 ml HNO3 +10 ml H2SO4) and 2 drops of HF. Boil gently until the ore is dissolved and then evaporate to fumes. Cool and dilute with 80 ml water and 4 ml 1.2 sp.gr HNO3. Raise to a boil and carefully add 2.5 gm solid ammonium per sulphate. Boil to precipitate any manganese present in the ore. Filter through a pulp filter, collecting the filtrate in a fall beaker wash thoroughly with hot water. To the filter add a little excess of ammonia and about 2 gm ammonia oxalate crystals. Boil until everything goes into solution and then add carefully 10 ml ammonium carbonate solution. Boil again allow to stand for some time and filter wash with hot water. Transfer the mixed

precipitate to a beaker and redissolve it in a minimum quantity of Hcl. Again make the solution oust ammonia cal and add 5 ml saturated ammonium oxalate and 10 ml ammonia carbonate solutions filter and wash. Repeat the last separation and wash the mixed precipitate thoroughly with hot water. Make the mixed filtrate just acidic by adding Hcl in a conical flask. Test with a litmus paper. Add 5 ml of saturated sodium phosphate solution and 50 ml of ammonia. Shake vigorously for 5 minutes. Cool and let stand for 30 minutes. Filter through a pulp filter and wash well with 5% ammonia. Dry the pulp with the precipitate and transfer to a silica crucible. Ignite slowly at the mouth of a muffle furnace until fully charred and then at a red heat. Coal and weigh the residue as Mg­2P2O7. From this, calculate the percentage of Mgo in the ore sample.

**Determination of total Sulphur:**

**Rapid determination:**

Weigh 1.0 gm ore sample and mix thoroughly with 0.2 gm each of Cao and MnO2, both free from sulphur. This addition may be omitted if the ore contains above 5% Cao. The mixture is ignited in a porcelain crucible in an electric muffle. Furnace at 5000C. For about 10 minutes cool and transfer the ignited sample to a 250 ml tall beaker. Add exactly 7 ml of 1.16 sp.gr Hcl. Cover with a watch glass and digest for 5 – 10 minutes on a hot plate. Dilute with about 10 ml hot distilled water and boil for a minute. Filter at once through a filter paper and wash properly 4 – 5 times with hot water, add 15 ml of a 10% BaCl2 solution and proceed exactly as set out in the previous case.

**Determination of Phosphorus Pentoxide:**

Dissolve 2 gm ore sample in 40 ml conc Hcl in a beaker and evaporate to dryness. Re dissolve in 20 ml conc. Hcl and dilute with 30 ml water. Filter the residue and wash properly. Reserve the filtrate. Ignite the residue in a platinum crucible and fuse with about 2 – 3 gm of Na­2CO3. Extract the fused mass with hot water. Filter again and add this filtrate to the previous one. (Fusion of the residue with Na2Co­3 is not necessary when the ore is free from TiO2) add 30 ml conc. HNO3 to the combined filtrate. Evaporate to low bulk & again add 30 ml of 1.2 Sp.gr. HNO3. Heat the solution to boiling & add a solution of KMno4 until a pink colour persists continue boiling for a few minutes & then destroy excess KMno4 with NaSO3. Boil 2 minutes longer cool to about 700C add 40 ml of ammonium molybdate solution. Shake vigorously for sometime filter wash the flask & filter paper a number of times with dil. HNO3 & then with a solution of KNO3. Until the precipitate is free from acid. Drop the filter paper with the precipitate into the original flask add a known quantity (10 – 15 ml) of standard NaOH solution from a burette into the flask & shake vigorously until the filter paper is converted into a pulp add 3 – 4 drops of phenol plethlein indicator & titrate the excess NaOH against a standard solution of HNO3 the number of milliliters of the standard NaoH consumed will give a measure of the phosphorus content in the ore from the amount of phosphorus, the percentage of P2OS in the ore can be calculated.

**22. Determination of Na+ and K+ in solution by flame photometry**

**Theory and background**

**Sodium and potassium**

The major cation of the extracellular fluidis **sodium**. The typical daily diet contains 130-280 mmol (8-15 g) sodium chloride. The body requirement is for 1-2 mmol per day, the excess is excreted by the kidneys in the urine.

**Reference range (intervals) for sodium**

* Serum 136-145 mM
* Cerebrospinal fluid 130-150 mM
* Sweat 10-40 mM
* Urine (varies with intake) 40-220 mmol/day

**Hyponatraemia** (lowered plasma [Na+]) and **hypernatraemia** (raised plasma [Na+]) are associated with a variety of diseases and illnesses and the accurate measurement of [Na+] in body fluids is an important diagnostic aid.

**Potassium** is the major cation found **intracellularly**. The average cell has 140 mM K+ inside but only about 10 mM Na+. K+ slowly diffuses out of cells so a membrane pump (the Na+/K+-ATPase) continually transports K+ into cells against a concentration gradient. The human body requires about 50-150 mmol/day.

**Reference range (intervals) for potassium**

* Serum 3.5-5.1 mM
* Cerebrospinal fluid about 70% of serum
* Sweat 4.0-9.7 mM (men) 7.6-15.6 mM (women)
* Urine (varies with intake) 25-125 mmol/day
* Erythrocytes (intracellular) 105 mM

**Hypokalaemia** (lowered plasma [K+]), **hyperkalaemia** (increased plasma [K+]) and **hyperkaluria** (increased urinary excretion of K+) are again indicative of a variety of conditions and the clinical measurement of [K+] is also of great importance.

***The flame photometer***

A traditional and simple method for determining **sodium** and **potassium** in biological fluids involves the technique of emission **flame photometry**. This relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionise, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. You are probably familiar with the fact that if you sprinkle table salt (**NaCl**) into a gas flame then it glows bright orange (**KCl** gives a purple colour). This is the basic principle of flame photometry. A photocell detects the emitted light and converts it to a voltage, which can be recorded. Since Na+ and K+ emit light of different wavelengths (colours), by using appropriate coloured filters the emission due to Na+ and K+ (and hence their concentrations) can be specifically measured in the same sample. One drawback of flame photometers, however, is that they respond linearly to ion concentrations over a rather narrow concentration range so suitable dilutions usually have to be prepared. They are also rather complex and relatively expensive machines, as you will see. A flame photometer can also be used to measure the element **lithium** in serum or plasma in order to determine the correct dosage of lithium carbonate, a drug used to treat certain mental disturbances, such as manic-depressive illness (bipolar disorder).

* In this practical you will calibrate a flame photometer using standard sodium and potassium solutions then measure the Na+ and K+ concentrations in a redissolved oral rehydration sachet.

**Reagents: oral rehydration sachet**

**NaCl standards: 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 ppm**

**KCl standards: 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 ppm**

**Procedure:**

1. Carefully open an **oral rehydration sachet** and empty the contents into a **clean** 250 ml beaker. Add about 150 ml distilled water and gently swirl the contents until dissolved.

2. Pour the solution into a 200 ml volumetric flask and rinse out the beaker with small amounts of distilled water, adding the washings to the flask. Finally, make up the flask to exactly 200 ml and mix thoroughly.

3. Make a 1/50 dilution of the redissolved sachet solution by accurately pipetting 2 ml of the solution into a 100 ml volumetric flask and making up to 100 ml with distilled water**.**  ***Instructions for use of the flame photometer:***

4. Ensure that the photometer drain is leading into a sink and that the instrument is connected to gas, air and electricity supplies. Ensure the mains supply gas tap is **off**.

5. Turn the "**Sensitivity**" and instrument "**Gas**" controls control fully counterclockwise (towards you).

6. Insert the **sodium** optical filter.

7. Switch on the instrument and unclamp the galvanometer by turning counterclockwise.

8. Open the mica window, turn on the mains gas supply, light the gas and close the window.

(**CAUTION: DO NOT LEAN OVER THE INTRUMENT OR YOU WILL SET YOUR HAIR ALIGHT**)

9. Turn on the air supply control and adjust the air pressure to 10 lb/in2. Leave for 1-2 minutes to stabilise.

10. Place a beaker of distilled water into position at the left hand side of the instrument and insert the narrow draw tube into it to allow water to pass through the photometer. (**NOTE: once** **set up, the photometer must have water running through it at all times when a salt** **solution is not being measured. The rate of uptake is fast, so make sure there is always enough water in the beaker**).

11. Adjust the gas control to give a flame with a large central blue cone then, with water passing through the instrument, **slowly** close the gas control until ten separate blue cones just form.

12. Set the galvanometer to zero using the "**Set zero**" control.

13. Replace the distilled water with the 5 mM NaCl standard and adjust the "**Sensitivity**" control till the galvanometer reads 100.

14. Quickly but carefully, replace the 5 mM NaCl standard with standards of decreasing concentration from 4 mM to 0.25 mM and note the readings in the Table below.

15. Run water through the instrument again for 1-2 min then place the draw tube into a beaker containing the **1 in 50 diluted** rehydration sachet solution and note the galvanometer reading.

16. Run water through the instrument again and replace the sodium with the **potassium** filter.

17. Repeat the above procedure with the KCl standards, setting to 100 with 2.0 mM KCl, then reading the others in reverse order. Then read the **1 in 50 diluted** rehydration sachet solution.

18. Finally, run water through the instrument until the flame appears free of colour again.

19. When the instrument is no longer required, switch off in the following sequence:

* **Turn off the gas control and the mains gas supply**
* **Wait for the flame to die out.**
* **Turn off the air supply.**
* **Switch off the electricity**

20. Plot the galvanometer readings against Na+ and K+ concentrations on the graph paper provided (separate graph for each ion) and from these **calibration curves** determine the Na+ and K+ concentrations in the diluted sachet solution. Finally, calculate the Na+ and K+ concentrations in the **undiluted** sachet solution.

23. The Calcium Content of Milk

How can we determine the calcium content of milk?

**Introduction**

Calcium has long been known to be necessary for a healthy diet. Dairy products are touted as an excellent rour.. of ralcium. In this experiment you will determine the amount of calcium in milk by gravimetric titration with EDTA. The calcium concentration in the milk will be determined by finding the mass of the EDTA solution needed to titrate a known mass of milk to a blue endpoint' This process is similar to the method often used to {ind the hardness of water which is caused primarily by magnesium and calcium ions. EDTA (ethylenediamine tetraacetic acid) is a large molecule that has a strong attraction for metallic ions like Ca2+,the form of calcium in milk. The calcium ions and the EDTA combine in a 1:1 mole ratio to form a large complex ion called a "chelate." Chelates, such as the hemoglobin contains the Fe2\* ions and is found in your blood cells, are common in nature' In this titration, you will carefully weigh a pipet full of EDTA solution, then add the EDTA dropwise to a known mass of milk. An indicator will be added to the milk to tell you when all the calcium ions have been removed from the milk. The indicator that you will use, hydroxy naphthol blue, changes from red, when calcium is present, to blue, when all of the calcium ions have been removed from the solution. As you add the EDTA the calcium ions will be used up, causing the indicator to change color. The blue end point appears gradually, so you may want to run a preliminary trial just to observe the color change before you do your quantitative measurements. Because the indicator only works when the system is basic, you will add adrop of 6-molar sodium hydroxide solution to each of yourtitration samples. Remember that red and blue make purple; the mixture will be purple when both the calciumcontaining form and the calcium-free form of the indicator are present in about equal amounts. This will occur before the blue endpoint. Using the mass of EDTA in your solution and the mass of the milk, you will be able to determine the number of milligrams of calcium in240 mL of milk (about one cup).

**Reagents**

* Milk; whole, 20%, skim
* EDTA solution, 1.00%
* 6 M NaOH
* hdroxy naphthol blue indicator

**Procedure**

1. Label three pipets: EDTA NaoH and Milk fill each with the appropriate solution.

2. Weigh the milk pipet and the EDTA pipet and record their masses in a Data Table' The mass of the NaOH pipet is not needed.

3. Add 15 to 20 drops of milk to one well of your 24 well plate. Reweigh the milk pipet and record its mass in your Data Table.

4. Add one drop of 6M NaOH to the milk in the well plate'

5. Add a very smal amount of the indicator to the mixture in the well plate. Only a few crystals are needed. Sii, tt . mixture. It should have a red to rose color. If you think the color is too light, add a few more crystals, but if you use too much, results will be inconsistent.

6. Add EDTA solution a drop at a time to the mixture in the well plate. As yoy add the EDTA" the calcium ions will be tied up, removing them from the milk solution and causing the color to change, first to purple, then to blue. Record the mass of the EDTA pipet after the solution has turned blue.

7, Carry out two additional trials. If the results do not show good agreement, run additional trials, as needed. If you have not achieved consistent results after 5 trials, consult your teacher.

**Analysis and Conclusions**

1. Complete the Analysis and Conclusions section for this experiment either on your Report Sheet or in your lab report as directed by your teacher

The concentration of the EDTA solution has been adjusted so that exactly 1 gram of EDTA solution will tie up exactly 1.08 mg of calcium. Calculate the number of milligrams of calcium present in each of your three titration samples. Show your work for the first iitration. If you carried out more than three titrations, base all your calculations on the three that show the best

agreement.

2. Use your results from the first calculation to determine the number of milligrams of calcium in I cup (-240 mL) of milk. Report both the individual values for the three samples and an ave.rrga value. Assume that skim milk has the same density as water. Show your \*ork for trial

3. Calculate the individual deviations from the average for each trial. Calculate the average deviation for your three trials.

4. When an average deviation is known for a series of analyses, the results of the analysis are generally given in the form: (average value) \* (average deviation). Report the concentration of calcium in milk with the average deviation.

5. Calculate the percent deviation for your experimental results. Show your work.

6. The USRDA for calcium is 1200 mg per day. What fraction of the daily requirement would one cup of milk provide, based on your average value?

7. According to the carton label, one cup of milk provides 35% of the USRDA for calcium. What is your percentage error, assuming the carton value is correct?

8. Women over the age of 50 and men over age of 65 need about 50% more calcium than younger adults. Why is this?

9. A student purchased a calcium supplement tablet which contained calcium carbonate. The student placed the tablet in water for several hours. Addition of NaOH and the indicator gave a blue solution.