CHAPTER III

MATERIALS

AND

METHODS
CHAPTER – III

MATERIALS AND METHODS

METHODOLOGY FOR THE TAXONOMIC STUDY OF AQUATIC ANGIOSPERMS:

In order to carry out the work systematically and maximum use of time plan of work have prepared. According to that plan my work consist three phases: (i) Field work (ii) Laboratory work and (iii) Compilation.

3.1 FIELD WORK :-

The results included in this thesis are based on the collection made from the selected area of the district almost for a period of two years. The work was done through well planed exploration covering all seasons. The aquatic flowering plants were collected from various sites of study area. The Anand district occupies an area 2951 sq km. The district is divided in total 8 Talukas.

For my botanical tour the entire area was divided in smaller groups, so that plant collection may be suitable and each group was visited every month. Practically almost every weekend was used for plant collection.

3.1.1 To make a list of floristic and medicinal aquatic angiospermic flora:-

The plan of my work include the survey of aquatic plants available in my study area. Along with this also collected semi aquatic plants generally found nearer to water bodies and on the banks of rivers and other water bodies. During collection spot photographs were also taken for each plant in different view. The close up of the twig covering leaves, flowers and/or fruits were also photographed. The flowering and fruiting season of different plants may vary so seasonal changes also affect the collection.

Generally the area receive rain almost in middle of June or in the beginning of July. after the first rain the plants need at least one or one and half month for their vegetative growth. So I started my field visit for plant collection in the beginning of July. Moreover the plants were collected in different stages of development. The plants are collected for making herbarium specimens. Before collecting the plants from the field, the data related to that species such as habit, habitat, color of flower, fruit etc were noted in field note. The data which cannot be discussed by observation of herbarium like presence of latex, significance odour of vegetative or reproductive parts of plant were also carefully noted. The medicinal value of aquatic plants was also noted.
which was provided by local people. The collected plants were preserved in the form of herbarium.

3.1.2 To document traditional and ethno-medical knowledge of aquatic angiospermic flora:
To document the traditional knowledge regarding with ethno medicinal aspects was provided by the local people belonging to those specific area. To fulfill this purpose I have arranged a meeting with old and knowledge local people. First they were not responding me about ethno-medicinal knowledge of plants, but later on they agreed when I told them that this is a part of my study and then they discussed about their knowledge. I also show them my collected plants and they told me their local name and the uses of such plants. I also become assure that which part of the plant is effective in the treatment of which disease from the information which they gave. They provide traditional knowledge about the plants regarding with the treatment in disease or any type of pain and how much dose should be give to cure it. This all information used in ethno medicinal uses.

3.1.3 To find out natural and anthropogenic threats to the aquatic angiospermic flora:
My study area is rich in vegetation. Numbers of water sources are also found which consist of aquatic flora. I found that Aquatic angiospermic flora is facing various threats which was the challenge for their survival in their habitats. Anthropogenic threats include human activities which may cause destruction of biodiversity. natural threats include mainly climate change, scanty rain, cattle grazing, over population, collection of fire wood, poaching, man-animal conflict, illegal and unscientific collection of plants for fodder from restricted area etc.

3.1.4 To study the commercially useful aquatic angiospermic flora:
During my study I surveyed local markets and found out the aquatic plants used for commercial purposes. All data obtained were gathered and find out the utilization of aquatic plants as for example lotus flowers are widely used in worship of Goddess Laxmi and it is sold at high cost in the market.

3.1.5 To find out area rich in aquatic angiospermic flora diversity:
During my work period the data of aquatic plants were collected from various sites. After compilation I came to know the area which is very rich in flowering plant diversity. Depending upon the greater number of plants available at the sites of study area recognized as richest area in aquatic plant diversity.
3.1.6 Study of physicochemical properties of water: -
Along with plant collection I have also collected water sample for analysis and to find out various physico chemical parameters. I have collected total 24 water sample from different water sources including ponds, lakes etc. I have collected water sample to the depth of ½ meter by avoiding surface water. Water sample were collected in plastic bottles. After collection soon the plastic bottles were numbered and named (site name). water analysis was made in the laboratory based on the methods given in “APHA”, “Hand Book of Methods in Environmental Studies” Vol -1 Water and Waste Water Analysis by S.K. Maiti and “Environmental science and biotechnology theory and techniques” by Murugesan, A. G. and Rajkumari, C. (2005).

3.1.7 Collection procedure: -
The collection of plants was made systematically. A healthy moderate sized twig with flowers or fruit should be collected. The collected specimen should have a clear phyllotaxy by which one can easily justify the family or genus, because specific phyllotaxy is the identity of specific families. Along with phyllotaxy the entire inflorescence should be collect if possible. Different plants have diverse texture in case of branch, stem, so according to that aids for cutting should be used. Such as herbs can be easily cut by normal cutter, whereas the specimen consist of somewhat hard stem then pruner is used to cut it. In case of spiny plant the leather hand gloves should be wear to avoid injury by spines. For marshy plants like fern digger is used to take out the underground plant specimen from the wet soil. The stem of some plant grow horizontally under the ground, such plants should be cleaned from soil particles. Collection of aquatic plants is more difficult than of other, because aquatic plants are floating or filamentous. It is also difficult to arrange them on sheet as the plants possess fragile stem and leaves. A sheet of bloating paper to be kept under floating or submerged plant specimens to allow the absorption of water then slowly raising the paper until the specimen is lying on the paper out of the water. Make a slope carefully, so that it facilitates water runoff. Then transfer the specimen in other bloating paper and keep it in small plastic bag away from other hard plant specimen to avoid damage. These plants can easily damaged by larger plants on the same collection site so they should be collected in separate bags within larger bags. During collection the care should be taken to avoid diseased plant specimens or infected part of plant. All collected plants should be grouped according to their location from where they are collected. It is not possible to identify all
collected plants in field, so they have been dried and pressed to store for long time. After further process of preservation the collected plants were brought to laboratory.

3.2 LABORATORY WORK:-

Laboratory work include preparation of Herbarium sheets. For this various techniques should be applied like field note, drying, pressing, poisoning, mounting, labeling etc.

3.2.1 Identification of Plants:-

Before the application of various herbarium techniques identification of plants is prime important. The procedure was carried out with the help of flora and the subject experts. Identification was done in two steps. Firstly, the specimens were cautiously identified by using regional floras such as G. L. Shah’s “Flora of Gujarat State”, and also other like Flora of Bombay presidency by Cook and Flora of North Gujarat by Saxton and Sedgwick. The final confirmation was done by matching with voucher specimens kept in herbarium of Sheth M.N.Science College, Patan. For this the collected plants were brought to the laboratory. Then all characters regarding with leaves, stem, flower and fruit were note down separately. Each part of the plants were examined for identification in fresh condition if possible. In case whenever the examination were done for dried plants, then the flowers were put in boiling water before sometime of dissection.

3.2.2. Herbarium Technique For Plant Preservation:-

Introduction:-

The plants preserved for very long time for further study by a definite technique in dry form is called herbarium. The collection of plants has began in the 16th century. Later, J.P. Tourefort (ca 1700, France) used the term herbarium for plants (Bridson and Forman 1999). Herbarium are fundamentals for taxonomic researches because they serve as voucher specimens. They play an important role in the identification of the family, genus and species. So the herbarium is basically recognized as a storehouse of botanical specimens, which are arranged in the sequence of conventional classification system. Which are available for reference or other scientific study. They are suitable for easy transfer from one place to another place for study. The plants which are mounted and deposited in herbarium are referred as herbarium specimens. The herbarium specimens can be store for many years, such herbarium serve as historical collection, reference collection for identification of newly collected plants and also serve as teaching aid as a source of research material. These are the reasons why the Taxonomic research include the collection of
plant which built up over a long period of time, a herbarium which proved as important collection material useful in research and for future study.

Herbarium is termed as ‘Dry garden’ which is used for further research work. Collector must learn how to make excellence specimens which will further utilized by Botanist for identification. The plants are collected in far-off places and sent thousands of miles away from collection site. It is also possible that study may be carried out after many years from collection. At the time of collection the collector should make a field note carries many detail that will be unknown for botanist who is going to study the plant. Some time the flowers or fruits may vanish during drying or transport, so at that time good photos would help in identification. The photographs should be taken with SLR camera. A collector should collect at least two specimens of each plant if it can not identified, one to be sent to a botanist for identification, so that the person who identifies them can keep one specimen in return for naming them. The specimen of the plant that first and originally receives the name becomes the "Type specimen" to which all others are compared. Wherever critical judgment is required, files of dried herbarium specimens are the only solution for identification.

(a) Materials required for plant collection.

- Plant cutter (secateurs), pruner, digger, knife, leather gloves.
- Flimsies or newspaper, blotters, corrugated plates, herbarium pressure, straps, tissue paper, plastic (poly) bags, herbarium bags.
- Drying table, mountain survival blanket, clipper, and heater.
- First aid box, topographic maps, binocular.

(b) Field note:-

After collection the records of field were recorded in small notebook, termed as field note. A ideal field note include various data such as date of collection, collection number, location, Longitude & Latitude of that location by GPS, name of specimen if possible, morphological description, color of flower or fruit which may change after drying. Field record is must for herbarium specimens without it the specimens would become worst. Floral characters proved as essential aid in identifying the species of plant. It is advisable to examine floral characters, if
they are small, should be observed with magnifying lens. Finally the distribution status of plant also needs to be mentioned, either the collected species is rare, frequent, common, locally common or occasional.

(c) **Pressing:**

After collection the next most usual method is pressing the specimens. For pressing, blotting paper sheets or old newspapers were used. Every care was taken to avoid deshaping and overlapping of plant parts. Sometime the specimens are collected in plastic bags and later on they will press. The specimens are kept smoothly within newspaper. Parts of flower should more carefully spread without overlapping in original shape. If the specimens are long, then it desires to be folded in V and N or Z shape. During pressing unnecessary overlapping leaves and other parts must be avoided. In case of large compound leaves it need to tear in half lengthwise and one half is removed. Some leaves should be turned over to show both dorsal and ventral surfaces. water plants or other plants with pseudobulb or fleshy rhizome requires to remove or cut by knife, so that moisture evaporates. Flowers with fused corollas few flowers should be pressed separately and some of these split open and spread. In case flower is large cotton padding is suitable make it dry very quickly. The specimens thus kept inside blotters, are covered by on either side by blotters and then it is put under herbarium pressure. After all the specimens pressed, the plant press is closed and pressure applied by means of tightening the straps. Hard and dried fruits and cones do not need press, but have to keep in special boxes.

(d) **Drying Techniques:**

Drying techniques are of two types; (1) Drying without applying heat and (2) Drying by applying artificial heat

1. **Drying without applying heat:**

The first method without applying heat is most convenient. In this method the plants are pressed in between the blotters. No corrugates are used. The press is locked up for about 24 hours; this is known as the sweating period. Then it is opened and the blotters are removed and all sheets turned back and after checking rearranged them as the earlier position. The blotters should be changed at regular interval, if they get moistened. And then the specimens are kept with the new blotters. The process of replacing blotters should be repeated and blotters must be changed at least 3-4 times for better result. Then the new stack of blotters and specimens is locked up in the press and allowed to stand for another 24 to 36 hours. Moreover for proper ventilation and
equal heat diffusion the aluminium plates of about 12 x 18 inch are placed. Then it is tied up inside the herbarium pressure by two belts around the outside. Usually they are made up of metal, plywood or wooden grid frame of 30 x 46 cm (Woodland, 1997). The herbarium pressure also can be prepared very cheaply by using wood straps, old cardboard boxes, old newspaper and rope. About a week is required for completion of drying. Dried specimens are packed with much care. Fungi as well as insects damage if proper care is not given till the permanent storage and plant specimens may be worst and useless.

(2) Drying by applying artificial heat:–
Whereas in second method drying with artificial heat is the prevalent method. In this method heat, dry air is passed through the canal of corrugate. Corrugates referred as ventilators used when plants are dried by applying artificial heat (Lawrence, 1991). It is as sheet of pasteboard or thin aluminium metal, with fluted ducts. It provides air passages through the press for movement of dry heated air. The dryer with the herbarium pressure is covered by mountain survival blanket. It is heat resistant blanket that is attached to stand with clips. Series ventilations should be keep at the base and at the tip, for coming and for outgoing air respectively. The best heat source is heater. In the absence of heater stove can be used as an alternative source. The usual time period for drying specimens is 12 hours. But it may be vary because it depends upon the material; and also dryer set, humidity, the type of heat source, climate, and temperature which affect the drying period. The time for drying must be maintained carefully, otherwise the specimens may be worst. The specimen become brittle and discolored at high temperature for too long a time period. Whereas at very low temperature for too short a drying period the specimens will remain moist and cause fungal infection. Specimens should be checked regularly until dry. The pressures loose when the plants are dry.

(e) Mounting herbarium specimens:–
Mounting is the process in which a specimen is attached to a herbarium sheet. Specimens are mounted on sheets of standard size herbarium paper (29 X 43 cm) and box size is 4 x 6 inch. North American standard size sheets are 29 x 41.5cm or 11.5 x 16.5 inch. (Woodland 1997). Most herbaria use a glue or fevicol as adhesive to fix the specimens to the sheets. The specimen may be attached by various methods. A common method involves smearing a glass plate with a
water-soluble paste, placing the specimen on the paste, and then transferring the glued plant to the mounting sheet. Small fragment packets or paper envelopes are attached to the sheet to hold seeds, extra flowers, or any part of the specimen.

(f) Poisoning:-
Poisoning is most essential for the formation of good and perfect herbarium. After cautiously drying, pressing, mounting, if proper poisoning was not done then you never make good and healthy herbarium. Precaution should be taken to protect herbarium specimens from damage by insects, pests and fungi. The most destructive insects are herbarium beetle, cigarette beetle, book-lice, and silverfish. To destroy these insects the insect repellents should be used. Insect repellents such as naphthalene ball or Para dichlorobenzene are sometimes placed in small quantities in herbarium cupboard. Although mercuric chloride is believed to be valuable because it provides long term protection against insect attack but remember that mercuric chloride is dangerous to health and may proved fatal. Besides the insect pest, the moulds and mildew are constant threat to material stored in moist condition or in high humidity areas. Naphthalene and LPCP are believed to have fungicidal properties; however, Thymol is quite effective as a fungicide.

(g) Label
Herbarium label is an important and essential part of permanent plant specimens. The size and shape of label may vary slightly but usually it will be a rectangular and size ranges between 10 x15 cm (4 x 6 in.). The location of label is also very important to decide where it should be placed. The best location for label is the bottom right side corner. Which makes the label suitable to read when kept in genus covers which open on the right hand side. Preferably a space should be left above the label to permit for the future attachment of determination slips. Generally herbarium label should contain the following information:-

- Heading:- Name of the institution where the specimens deposited.
- Scientific name:- Genus, species, specific nickname of author or authors.
- Family:-
- Locality:-
- Habitat:-
3.3. **COMPILATION**:-

This phase includes updating of nomenclature according to the rules laid down by “International Code of Botanical Nomenclature (Vienna Code, 2005)” preparation of keys to genera and species and the treatment of individual families. To accomplish this latest names appearing in recent monograph, papers concern with nomenclature published in standard journal and latest floras have been adopted. Keys given in systematic portion are artificial and of dichotomous type. It has been prepared in such a way that it to be proved as supplements to those as was in original floras. Only those characters have been taken which could be easily observed, with the help of a hand lens.

All families have been arranged according to the system of Bentham and Hooker with minor modifications. This system has been adopted because of its practical merit and its popularity and wide spreading in India. Each family possessing a key to genera followed by more than one species. It also having correct name a short synonym, brief description, flowering and fruiting period and lastly the place of collection and status.

3.4 **METHODOLOGY FOR WATER ANALYSIS**:-

3.4.1 **SAMPLING METHOD**:-

The study was carried out for 24 different water bodies which include ponds and lakes of Anand district. Total 24 water bodies were selected for water analysis from different Talukas of Anand district. Total three ponds were prefered from each Taluka. Anand district comprises 8 Talukas and so on total 24 study sites were selected for collection of water sample to study physico-chemical analysis of water. For better result proper sampling method was necessary. During the present study the following described points and methods were kept in mind to get accurate and proper results.

3.4.2 **SELECTION OF SITES FOR WATER SAMPLE COLLECTION**:-
In the present study total 24 water bodies were selected for analyses the water quality. Water samples were collected during early morning hours to get proper results. The samples were collected from four different points of ponds such as inlet point, outlet point, center point and remaining point is selected randomly. Here four points were selected to get accurate result of water quality of entire pond or lake. Proper sampling method was used because appropriate sampling is a pre condition for good analysis.

3.4.3 SAMPLE COLLECTION METHOD:-

Water sample collection time was preferred early morning hours. The water samples were collected in plastic bottles or in glass bottles. Before collection the bottles were carefully rinsed with sample water. To collect sample first of all closed bottle was dipped in pond or lake up to the depth of about ½ meter from the water surface, and then bottle was opened inside the water and soon closed after collection to prevent surface water. The water samples were collected from four different points and the all four samples were mixed together to prepare an integrated water sample which is desirable for proper analysis.

3.4.4 SAMPLE PRESERVATION METHOD:-

After collection water samples should be carefully preserved, because in between the time of water sample collection and analysis a number of physical, chemical and biological reactions occur in water, which would change the quality of water sample. Therefore to avoid or minimize such changes the water sample were preserved soon after collection. The water samples were preserved by adding chemical preservatives, lowering the temperature or by both the methods which is suitable. The parameters like temperature, pH, DO, TDS were analyzed at the time of collection while the other parameters were done in laboratory.

The study was carried out for a period of 1 year from Nov 2012 to Nov 2013. After collection soon the water samples were brought to the laboratory for the further experimental analysis. Among these pH was determined by using digital pH meter, Electrical conductivity (EC) was measured by electrical conductivity meter. Initially DO was measured by DO meter and later on it was also determined by the method as given in APHA. Then total dissolved solid was measured by TDS meter and turbidity was measured by nepheloturbidity meter. Whereas other parameters like alkalinity, chloride, total hardness, calcium were done by titration, sodium
and potassium were done by Flame photometer, phosphate was done by UV visible spectrophotometer. These all parameters were measured by applying the various methods of APHA.

3.5. METHODS FOR WATER SAMPLE ANALYSIS:-

In order to determine various physical and chemical characteristic water samples were collected from selected study sites of study area. Total 24 water sample were collected for physico-chemical analysis of water to determine the quality of water. Water samples were collected from study area such as ponds, lakes, puddles of Anand district. Water samples were collected in plastic bottles. Soon after collection water samples were measured for pH, TDS, DO and other physical parameters. Then samples were filtered and preserved as above methods. Each bottle was labeled carefully. Then water samples were brought to the laboratory for the further analysis. Water samples were analyzed for various physical and chemical parameters like pH, Turbidity, EC, TDS, Cations (Ca$^{+2}$, Mg$^{+2}$, Na$^{+}$ and K$^{+}$), Anions (CO$_3^{-1}$, HCO$_3^{-1}$, Cl$^{-1}$ and PO$_4^{-1}$) and Gasses (COD, BOD, DO, FCO$_2$). The methods used for analysis of water parameters are as follows.

3.5.1 INSTRUMENTAL ANALYSIS:-

(a) pH:

pH is the measure of relative acidity or alkalinity of water. pH measures the concentration of hydrogen ions in water and it is expressed as negative logarithm of hydrogen ion concentration. If the value of pH found 0 to a little less than 7 is termed as acidic and the value of pH found little above in between 7 to 14 determine basic. 7 pH indicate the neutrality of water i.e. H$^{+}$ and OH$^{-}$ ions are equal.

Principle:- The pH electrode is used to measure pH. It consist of sensing half cell and reference half cell forming an electrode system. An electrical potential is developed inside and another developed outside, the difference is measured, which is known as pH of the sample.

Apparatus Required:- pH meter, Standard flasks, Magnetic Stirrer, Funnel, Beaker, Wash Bottle, Tissue Paper, Forceps

Chemicals Required: Distilled water, 4.01 pH tablet, 7.0 pH tablet, 9.2 pH tablet.
Preparation of Reagents:-

(1) Buffer Solution of pH 4.01:-
- Take 100 ml of standard flask and put funnel over it.
- Transfer one buffer tablet of pH 4.01 to the funnel by forceps.
- Crush the tablet and dissolve it in distilled water.
- Make the total volume 100 ml by adding distilled water.

(2) Buffer Solution of pH 7.0
- Procedure as above, use tablet 7.0

(3) Buffer Solution of pH 9.2
- Procedure as above, use tablet 9.2

Instrument Calibration:-
- The combination electrode was cleaned with distilled water.
- Electrode connected with pH meter.
- In 100 ml beaker take 9.2 P^H solution and place it on magnetic stirrer, by inserting teflon coated stirring bar and stir well.
- The electrode dipped in standard solution of 9.2 pH.
- If the instrument is not showing pH value of 9.2, adjust reading to 9.2 by using the calibration knob.
- Take out the electrode from buffer, wash it with distilled water and then wipe with soft tissue.
- Follow the same procedure for pH buffer 4.01 and 7.0
- Now the instrument is calibrated.

Standardize pH meter with three pH buffer solution. Then take about 30 ml of water sample in beaker and dipped the electrode and record P^H value. (Chaudhary et al., 1992).
PROCEDURE CHART:

1. Switch on the pH meter atleast 30 minutes before the test.
2. Calibrated pH meter by above methods
3. Electrode made dry by soft paper
4. Half of the electrode dipped into sample water.
5. pH value was recorded

(b) ELECTRICAL CONDUCTIVITY (EC):-

EC was measured by conductivity meter, Model: EQ-660A. The measurement unit of conductivity is mhos/cm.

Principle:-

Conductivity is defined as the ability to conduct heat, sound or electricity. It can be expressed as mhos. The conductivity of water is a measure of the ability of water to carry an electric current. Water generally consist of various kinds of ions which help in passing the electric current through water. Conductivity is dependent upon concentration of ions. Conductivity meter consist of conductance cells with platinum electrode. As ionization dependent on temperature, the temperature was maintained at 25°C.

Apparatus Required: Electrical Conductivity meter with electrode, Standard flask, Beaker, Funnel, Tissue paper, weight meter, spatula.

Chemicals Required:- Distilled water and 0.01 N KCl.

Instrument Calibration:-
Switch on the instrument before 30 minutes
Take 0.1 N KCl in beaker.
Switch on magnetic stirrer and put beaker on it.
Put the electrode in solution.
By using calibration button adjust it till the digital display show 1.000
Now the instrument is calibrated and ready to measure the sample

Sample handling and preservation:- sample water was collected in plastic or glass bottle. Analysis should be done soon after collection, if it can not done within 12 hours then sample should be preserved at 4°C. Freezing or chemical preservation is not required.

(c) TURBIDITY:- Turbidity indicate the presence of isolated and suspended solids like algae, clay, silt, organic matter and also microorganisms. Turbidity of water sample was measured by Digital Turbidity meter, Model: LT-33.

Procedure Chart:

1. Switch on Conductivity meter 30 minutes before.
2. Prepare 0.1 N Potassium Chloride solution.
3. Calibrate the conductivity meter using the standard solution of 0.1 N KCl by adjusting calibration knob.
4. Temperature was adjusted at 25°C.
5. Dip the conductivity cell in water sample.
6. Record the reading in mho/cm unit.

Principle:- Turbidity is based on the comparison of light intensity scattered by sample with that of light intensity scattered by standard suspension. Thus turbidity of given sample is measured from amount of light scattered by sample with standard turbidity suspension. i.e. if the scattered light intensity is higher, so the turbidity is high. Formazin polymer is used as the primary standard reference suspension. The turbidity of a particular concentration of formazin suspension is defined as 4000 NTU.
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**Apparatus Required:-** Digital Turbidity Meter, Sample Cells, Standard flasks, Funnel, Wash Bottle, Tissue Papers

**Chemicals required:-** Distilled water, Hydrazine Sulphate, Hexamethylene Tetramine.

**Instrument Calibration:-**

- In the sample cells a first add distilled water up to horizontal mark and wipe with soft dry tissue.
- Place it in turbidity meter in such a way that sample cell should concur with the mark in the turbidity meter and cover the sample cell.
- Now adjust the reading to zero by using the set zero knob
- Prepare standard solution.
- Add standard solution to the sample cells up to horizontal mark and wipe gently with soft dry tissue.
- Place it in turbidity meter up to vertical mark and cover the sample cell.
- If the instrument is not showing 4000 NTU then, adjust the instrument set as 4000 NTU by using the calibration knob.
- Now the instrument is calibrated and ready for use.

**Preperation of Reagents:-**

1. **Hydrazine Sulphate Solution:-**
   - Take 1 gm of hydrazine sulphate and dissolve it in distilled water.
   - Transfer it to 100 ml flask to prepare up to 100 ml of solution by adding distilled water.

2. **Hexamethylene Tetramine:-**
   - 10 gm hexamethylene tetramine and dissolve it in distilled water.
   - Transfer it to 100 ml flask to prepare up to 100 ml of solution by adding distilled water.

3. **Standard 4000 NTU Solution:-**
   - Mix 5 ml of solution (1) and 5 ml of solution (2) in 100 ml measuring flask.
   - Allow the mixture for 24 hours.
   - After 24 hours, make up the total volume 100 ml adding distilled water.
   - Standard 4000 NTU solution is ready.
Procedure chart:-

1. switch on turbidity meter 30 min before the test.

2. Calibrate the instrument by using standard 4000 NTU suspension

3. Calibrate turbidity meter to 0 NTU using distilled water.

4. Insert the sample in sample tube up to horizontal mark and put it in turbidity meter up to vertical mark

5. Note the reading in NTU unit

(d) **TOTAL DISSOLVED SOLIDS (TDS)**:- Total dissolved solids are the solids present in water in dissolve condition. They are determined as residue remain left after evaporation of filtered water at about 103°C. It is measured by digital TDS meter.

**Principle**:- The TDS meter is electrically charge mater in which two electrodes are equally placed which conduct a charge on inserting in water. The results are interpreted by instrument and converted into ppm. In case of water is pure then it will not conduct a charge and show 0 ppm. On the other hand if water contain much dissolve material, it will conduct a charge and resulted in ppm, relative to the amount of dissolve solids. This is due to all dissolve solids containing an electric charge, which permit electrical charge between the electrodes.

**Apparatus Required**:- Digital TDS meter, Beaker 100mL, Distilled water, Tissue paper.

Before starting the experiment calibration of instrument is required.

**Instrument Calibration**:-

- First start the instrument and put the lid in beaker containing distilled water.
➢ Note display reading zero, if the zero is not there set zero by set zero knob.

**Procedure chart:-**

- Immerse the TDS meter in water sample
- Wait till the stable reading
- Reading was noted in ppm

**(e) SODIUM (Na⁺):**

**Introduction:-**

The salts of Sodium are highly soluble in water. Generally it is found in lower concentration than calcium and magnesium in fresh water. The concentrations of Na⁺ may vary from 1 mg/L to more than 500 mg/L. In ground water it ranges from 6 to 13 mg/L, whereas in surface water it may be less than 1 mg/L or may exceed 300 mg/L depending on geographical area. The ratio of sodium to total cations is important in agriculture and physiology of human. High sodium ratio can damage soil permeability. High concentrations of sodium may cause cardiac difficulties in persons. A limiting concentration of 2 to 3 mg/L is recommended in feed waters destined for high-pressure boilers. When necessary, sodium can be removed by the hydrogen-exchange process or by distillation. The U.S. EPA advisory limit for sodium in drinking water is 20 mg/L.

**Principle:-**

Trace amounts of sodium can be determined by flame emission photometry at a wave length of 589 nm. Sample is nebulized into a gas flame carefully, which become colored according to the characteristic discharge of the metal. reproducible excitation conditions. The sodium resonant spectral line at 589 nm is isolated by interference filters or by light-dispersing devices such as prisms or gratings. Emission light intensity is measured by phototube, photomultiplier, or photodiode. The light intensity at 589 nm is roughly proportional to the sodium concentration. Alignment of the wavelength dispersing device and wavelength readout may not be accurate. The appropriate wavelength setting, which may be slightly more or less than 589 nm, can be determined from the maximum emission intensity when aspirating a sodium standard solution, and then used for emission measurements. The calibration curve may be linear but has a tendency to level off or even reverse at higher concentrations. Work in the linear to near-linear range.
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Apparatus Required:- Flame photometer operating in the flame emission mode.

Sample Handling And Preservation:-
To minimize sodium contamination, store all solutions in plastic bottles. Use small containers to reduce the amount of dry element that may be picked up from the bottle walls when the solution is poured. Shake each container forcefully to wash accumulated salts from walls before pouring solution.

Chemicals Required:- Stock Sodium Solution, Intermediate Stock Solution, Standard Sodium Solution.

Preparation Of Reagents:-
(1) Stock Sodium Solution:-

➢ Dissolve 2.542 g NaCl to 1000 mL with water; 1.00 mL = 1.00 mg Na.

(2) Intermediate Stock Solution:-

➢ Dilute 10.00 mL stock sodium solution with water to 100.0 mL; 1.00 mL = 0.10 mg Na
(1.00 mL = 100 µg Na).
➢ Use this intermediate solution to prepare calibration curve in sodium range of 1 to 10 mg/L.

(3) Standard Sodium Solution:-

➢ Dilute 10.00 mL intermediate sodium solution with water to 100 mL; 1.00 mL = 10.0 µg Na.
➢ Use this solution to prepare calibration curve in sodium range of 0.1 to 1.0 mg/L.

Instrument Calibration:-

➢ Start the instrument before 1 hour of analysis, then standardize it with stock solution.
➢ Prepare a blank and sodium calibration standards in stepped amounts in any of these applicable ranges: 0 to 1.0, 0 to 10, or 0 to 100 mg/L.
➢ Determine emission intensity at 589 nm.
➢ Construct a calibration curve from the Sodium standards and determine sodium concentration of sample from the calibration curve.
➢ Where a large number of samples run regularly, the calibration curve provides sufficient precision.
PROCEDURE CHART:-

1. first calibrate the instrument then start examination of water sample
2. Take the water sample in the curette and put it for analysis
3. Note down the reading.

(f) POTASSIUM (K⁺):

Introduction:-

Potassium is a vital constituent in both plant and human nutrition. It occurs in ground waters as a result of mineral dissolution, from decomposing plant material, and from agricultural runoff. The common aqueous species is K⁺. It does not remain in solution, but is assimilated by plants and is integrated into clay-mineral structures. Potassium is the fourth element in Group IA of the periodic table; it has an atomic number of 19, an atomic weight of 39.10, and a valence of 1. The average profusion of K in the earth’s crust is 1.84%; in soils it has a range of 0.1 to 2.6%; in streams it is 2.3 mg/L, and in groundwaters it has a range of 0.5 to 10 mg/L. Potassium compounds are used in glass, fertilizers, baking powder, soft drinks, explosives, electroplating, and pigments.

Apparatus Required:- Flame photometer operating in the flame emission mode.


Preparation Of Reagents:-

(1) Stock Potassium Solution:-

➢ Dissolve 1.907 g KCl dried at 110°C and dilute to 1000 mL with water; 1 mL = 1.00 mg K.

(2) Intermediate Potassium Solution:-

➢ Dilute 10.0 mL stock potassium solution with 100 mL water, 1.00 mL = 0.100 mg K.
➢ Use this solution to prepare calibration curve in potassium range of 1 to 10 mg/L.

(3) Standard Potassium Solution:-
Dilute 10.0 mL intermediate potassium solution with water to 100 mL; 1.00 mL = 0.010 mg K.

Use this solution to prepare calibration curve in potassium range of 0.1 to 1.0 mg/L.

**Instrument Calibration:** Instrument calibration method is same as in case of Sodium. Use Potassium Standard instead of Sodium Standard.

**PROCEDURE CHART:**

1. first calibrate the instrument then start examination of water sample
2. Take the water sample in the cuvette and put it for analysis
3. Note down the Potassium concentration.

**(g) PHOSPHATE \((PO_4^{3-})\):**

**Introduction:**
Phosphate is not poisonous to animals or plants. Phosphate present in water bodies is derived from pollution from fertilizer application, sewage and industrial waste. In fact, it is a plant nutrient which stimulates the growth of aquatic weeds and algae. This may cause lakes and rivers to become blocked and overrun with plants. Large amount of phosphate in water indicate pollution through sewage and industrial waste.

**Principle:**
The principle of this method involves the formation of molybdophosphoric acid, which is reduced to the intensely colored complex, molybdenum blue. This analytical method is extremely sensitive and is reliable down to concentrations below 0.1 mg phosphorus per liter.

**Apparatus Required:** The UV visible spectrophotometer will be employed in the measurement of color intensity of the blue solutions. A wavelength of 650 nanometers will be used in these analyses.

**Chemicals Required:** Ammonium molybdate Stock Phosphate Solution, Stannus Chloride Solution, Glycerol, Conc H$_2$SO$_4$

**Preparation Of Reagents:**

1. Ammonium Molybdate Reagents:
Dissolve 2.5g of ammonium molybdate in 17.5 mL distilled water.

Then carefully add 28 mL of Conc H\textsubscript{2}SO\textsubscript{4} to 40 mL of distilled water.

Cool it, add molybdate solution and dilute entire solution to 100 mL

(2) Stannus Chloride Solution:-

Dissolve 2.5 g of SnCl\textsubscript{2}•2H\textsubscript{2}O in 100 mL of glycerol.

Heat in water bath and stir with glass rod to hasten dissolution.

(3) Stock Phosphate Solution:-

Dissolve 0.286 gm of anhydrous KH\textsubscript{2}PO\textsubscript{4} in 1.0 liter water.

This is a 200 mg/L stock solution. Then dilute 100 mL of this solution to 1.0 liter.

PROCEDURE:-

Prepare the following standard Phosphate solutions:

1.0 mg/L standard: Place 2.00 mL of 20.0 mg/L phosphate solution in a 100-mL graduated cylinder and dilute to 40 mL with purified water.

2.0 mg/L standard: Place 4.00 mL of 20.0 mg/L phosphate solution in a 100-mL graduated cylinder and dilute to 40 mL with purified water.

3.0 mg/L standard: Repeat the directions for the 1.0 mg/L standard using 6.00 mL of 20.0 mg/L phosphate.

4.0 mg/L standard: Repeat the directions for the 1.0 mg/L standard using 8.00 mL of 20.0 mg/L phosphate.

5.0 mg/L standard: Repeat the directions for the 1.0 mg/L standard using 10.00 mL of 20.0 mg/L phosphate. (Save 25mL)

Blank: Take 25 mL of purified water which will be treated with the color developing reagent to serve as a blank solution. Then after these five standard solutions and the blank should be treated according to the following "color development" procedure. After measuring the absorbance of these solutions, make a plot of absorbance versus concentration.

Color development in sample:
This procedure is used for the five standard solutions and for water samples of any river, lake, or sewage which are to be analyzed for phosphate. Place 25 mL of water sample in an Erlenmeyer flask. Put 1.00 mL of ammonium molybdate solution into the flask and spin to mix. Then add 2 drops of stannous chloride solution to the same flask and mix by swirling. If phosphate is present, a blue color will develop in 5 minutes.

Note: The time period is somewhat critical. After addition of stannous chloride measurements should be taken anywhere within 5 to 15 minutes. While you are waiting to develop a blue color, set the wavelength to 650 nm on the spectrophotometer.

Use the blank solution to set it to read zero absorbance. Using 650 nanometers wavelength, measure the absorbance (after 5-10 minutes color development) of the blue sample.

3.5.2 CHEMICAL ANALYSIS:-

(a) TOTAL ALKALINITY (CARBONATE AND BICARBONATE):-

Introduction:

Alkalinity is a way of measuring the strong acid neutralizing capacity of water. Its capability to maintain constant pH is because of hydroxyl, carbonate and bicarbonate ions of water. The alkalinity of natural waters is primarily due to the salts of weak acids, and weak or strong bases, and substances act as buffers. Bicarbonate represents the major form of alkalinity, formed from the action of CO$_2$ on basic materials in soil.

Carbonate : The phenolphthalein alkalinity represents all the hydroxide alkalinity plus one-half the carbonate alkalinity. Therefore, carbonate alkalinity may be calculated as below. Carbonate alk. = 2 (phenol. alk. – hydroxide alk.). Bicarbonate: The titration from pH 8.3 to pH 4.5 measures the remaining one-half of the carbonate alkalinity plus all the bicarbonate alkalinity. Bicarbonate alk. = Total alk. – (carbonate alk. + hydroxide alk.).

Principle: - The alkalinity of water can be find out by titrating water sample with a strong acid H$_2$SO$_4$ to known value of pH. When a water sample with pH greater than 4.5 is titrated with acid to a pH 4.5 end point, all CO$_3^{2-}$, HCO$_3^-$ and OH$^-$ will be neutralised. For pH more than 8.3 using phenolphthalein as indicator, the color changes to pink indicating the presence of hydroxyl ions (OH$^-$). On adding mix indicator the presence of CO$_3^{2-}$, HCO$_3^-$ change the color to blue. When
titrate with $\text{H}_2\text{SO}_4$ the blue color again changes into red, which indicate that $\text{CO}_3^{2-}$, $\text{HCO}_3^-$ become neutralized.

**Apparatus Required:** Burette with Burette stand and Porcelain Tile, Pipettes with elongated tips, Pipette bulb, conical flask (Erlenmeyer flask), 250 mL Measuring cylinders, Standard flask, Wash Bottle, Beakers.

**Chemicals Required:** Standard Sulphuric acid, Phenolphthalein indicator, Methyl orange indicator, Distilled water, Bromocresol green, Ethyl alcohol.

**Preperation of Reagents:**

(1) $\text{H}_2\text{SO}_4$ solution (0.02 N):

- Take around 500 ml of distilled water.
- Pipette 20 ml of concentrated 0.1 N Sulphuric acid and add slowly along the sides of the flask.
- Make up the total volume up to 1000 ml.
- This prepared solution is 0.02 N $\text{H}_2\text{SO}_4$ solution.

(2) Phenolphthalein indicator:

- Take 1gm and then add 100 ml of 95 % ethyl alcohol or 100 ml of distilled water.

(3) Mixed indicator:

- Take 100 mg Bromocresol green and 20 mg of methyl red and dissolve in 100 ml of 95 % ethyl alcohol or 100 ml of distilled water.

**Calculation for alkalinity:**

\[
\text{PA as CaCO}_3 \text{ mg/l} = \frac{(A \times \text{Normality}) \times 1000 \times 50}{\text{Volume of sample taken}}
\]

\[
\text{TA as CaCO}_3 \text{ mg/l} = \frac{(B \times \text{Normality}) \times 1000 \times 50}{\text{Volume of sample taken}}
\]
Where:-

A = Total ml of H$_2$SO$_4$ used with only Phenolphthalein.

B = Total ml of H$_2$SO$_4$ used with phenolphthalein and methyl orange indicator.

PA = Phenolphthalein alkalinity and TA = Total alkalinity.

**PROCEDURE CHART:-**

1. Fill the burette with Sulphuric acid
2. Take 100 ml of the water sample in flask
3. Add 2 drops of phenolphthalein indicator.
   - The solution remain colorless
   - Phenolphthalein Alkalinity (PA) = 0
   - Add 2 drops of methyl orange indicator
   - Titrate it against H$_2$SO$_4$ and continue titration till the color change from yellow to red
   - The color of solution changes to pink.
   - Continue titration with Sulphuric acid till the color disappears
   - The color disappears at end point
   - Calculate the alkalinity of sample as reading A


(b) **TOTAL HARDNESS** \((Ca^{2+} + Mg^{2+})\):-

Total hardness of water is a measure of total concentration of the calcium and magnesium ions expressed as calcium carbonate in mg/L. The water which has high mineral content is known as Hard Water. Hard water contain chlorides, bicarbonate and sulphates of calcium and magnesium. There are two types of hardness Temporary and permanent. Among them temporary hardness because of the existence of bicarbonates of calcium and magnesium. It can be easily removed by boiling of water. Whereas permanent hardness is due to the existence of chlorides and sulphates of calcium and magnesium, which cannot be removed by ordinary boiling of water.

**Principle:-** A water sample taken in conical flask. When a very small amount of indicator dye such as Erichrome Black T (EBT) added in a solution containing calcium and magnesium ions with 10.1 pH, the colour of solution turns into wine red. EDTA , the titrant when added, complexes with calcium and magnesium ions. When all calcium and magnesium complexed with EDTA and resulting the colour of solution from wine red to blue. Which is the end point of titration.

**Apparatus Required:-** Burette with Burette stand and porcelain tile, Pipettes with elongated tips, Pipette bulb, conical flask, 250 mL graduated cylinders, Standard flask, Wash Bottle, Beaker.

**Chemicals Required:-** Ammonium Chloride, Ammonium Hydroxide, Disodium Salt of EDTA, Erichrome Black T, Magnesium Sulphate.

**Preparation of Reagent:-**

(1) **Buffer Solution:-**

- Weigh 1.179 gm of EDTA in 50 ml distilled water and dissolve it thoroughly.
- Add 16.9 gm of ammonium chloride in the content of beaker and dissolve it.
- Weigh 780 mg of magnesium sulphate and transfer in beaker and dissolve it.
- Add 143 ml of ammonium hydroxide to the content of beaker.
Make the total volume of the content upto 250 ml by adding distilled water.
This buffer solution is used to maintain the pH of water sample in between 9 to 10.

(2) Erichrome Black T:-
- Weigh 0.5 gm of Erichrome Black T.
- Add distilled water and make final volume exactly 100 ml.
- Shake the content well and transfer it to clean bottle and named as EBT.

(3) Standard EDTA Solution: (0.02 M) :-
- Take 3.723 gm of EDTA sodium salt.
- Transfer the content to 1000 ml flask.
- Fill the flask with distilled water to make the total volume 1000 ml.

PROCEDURE CHART:-

1. Fill the burette with 0.02 M EDTA
2. Pipette out 20 ml of water sample in conical flask
3. Add 2 ml of Ammonia Buffer solution
4. Add 2 drops of EBT indicator
5. Titrate the content against standard EDTA solution
6. Continue the titration till the color turns to steel blue
7. Calculate the Total hardness

Calculation for Total Hardness:-

\[
\text{Total hardness mg/l as CaCO}_3 = \frac{\text{Volume of EDTA} \times N \times 50 \times 1000}{\text{Volume of sample}}
\]
Where :- \( N \) = Normality of EDTA

(c) **CALCIUM (Ca\(^{++}\)):-**

Calcium is usually found in highest concentration in all natural water. Its presence in water results from the deposition of limestone, gypsum etc. Calcium forms insoluble salts with soap and decrease the cleaning efficiency of soap.

**Principle:-** The calcium quantity in water is determined by titration of water sample with standard EDTA. An indicator ammonium purpurate is used because it combines only with calcium. The indicator gives pink color while calcium and magnesium ions have not complexed with EDTA. It gives color change from pink to purple when all calcium complexed.

**Apparatus Required:-** Burette with Stand & Porcelain tile, Pipettes with elongated tips, conical flask 250 mL measuring Cylinder, Standard Flask, Beakers, Wash bottle

**Chemicals Required:-** Ammonium purpurate, Sodium Chloride, Sodium Hydroxide, EDTA.

**Preparation of Reagent:-**

(1) **Standard EDTA Solution: (0.02 M) :-** (Same as in Total Hardness).

(2) **Ammonium purpurate:-**

- Take 0.5 gm of Ammonium purpurate and transfer it to dry beaker.
- Weigh 100 gm of Sodium chloride and transfer it to the same beaker having Ammonium purpurate.
- Mix the contents and use it as dry powder.

(3) **1 N Sodium Hydroxide solution:-**

- Take 100 ml of distilled water in beaker.
- Weigh 4 gm of Sodium Hydroxide powder and transfer it to beaker containing distilled water and mix it thoroughly.
- Transfer the entire content to standard 100 ml flask.
- Make the final volume 100 ml up to the mark by adding distilled water.

**PROCEDURE CHART:-**

1. Fill the burette with EDTA
2. Pipette out 20 ml of water sample
3. Add 2 ml of Sodium Hydroxide
Calculation for Calcium Hardness:

\[
\text{Calcium hardness mg/l as } \text{CaCO}_3 = \frac{\text{Volume of EDTA} \times N \times 50 \times 1000}{\text{Volume of sample}}
\]

Where:

N = Normality of EDTA

\(d\) CHLORIDE (Cl\(^{-}\)):

Chlorides are generally distributed as salts of calcium, potassium and sodium in fresh water or wastewater. The salty taste in water is because of chloride anions and related cations. Sodium chloride and calcium chloride are present as major taste producing salts in water.

Principle: The concentration of chloride is measured by titrating the sample with silver nitrate solution. Silver nitrate reacts with chloride ion. Silver chloride is precipitated quantitatively before the formation of red silver chromate indicating the end point of titration. The results are expressed in mg/l.

Apparatus Required: Burette with Burette stand and porcelain tile, Pipettes with elongated tips, Conical flask, Standard flask, Beaker, Wash bottle.
**Materials and Methods**

**Chemicals Required:** Silver nitrate, Phenolphthalein indicator, Sodium chloride, Potassium chromate.

**Preparation of Reagent:**

1. **Standard Sodium Chloride Solution:**
   - Weigh 1.648 gm of Sodium Chloride. Transfer it to beaker containing distilled water and dissolve it thoroughly using glass rod.
   - Make the total volume of the solution of 100 ml by adding necessary distilled water.

2. **Standard Silver Nitrate solution (0.0282 N):**
   - Weigh 4.791 gm of silver nitrate and transfer it into beaker containing 100 ml distilled water.
   - Store the solution in yellowish-brown bottle.

3. **Potassium chromate indicator:**
   - Take 25 gm of Potassium Chromate. Transfer it to the beaker with distilled water.
   - Add few drops of Silver Nitrate in the solution till the formation of slight red precipitation.
   - Allow the content for 12 hours.
   - After 12 hours filter the solution with filter paper and then dilute the content to 1000 ml by using distilled water.

**PROCEDURE CHART:**

1. Pipette out 20 ml of water sample
2. Add 1 ml of Potassium chromate indicator
3. Fill the burette with Silver nitrate (stored in brown bottle)
4. Titrate the content against Silver nitrate solution till the color changes from yellow to brick red

**Calculation for Chloride:**

\[
(V_S - V_B) \times N \times 35.45 \times 1000 = \text{Chlorides mg/l} \]

\[
\text{Chlorides mg/l} = \frac{(V_S - V_B) \times N \times 35.45 \times 1000}{\text{Volume of sample}}
\]
Where:-

\[ V_S = \text{Volume of Silver Nitrate for Sample} \]

\[ V_B = \text{Volume of Silver Nitrate for Blank} \]

\[ N = \text{Normality of Silver Nitrate} \]

35.45 = Equivalent weight of Chlorine

3.5.3 GASSES ANALYSIS:-

(a) Dissolved Oxygen (Do):-

Introduction:-

Dissolved Oxygen is the amount of Oxygen dissolved in a unit volume of water. It is important for the maintenance of ecosystem of water bodies and also to keep the lakes and rivers healthy. The minimum DO level of 4 to 5 mg/L is desirable to continue existence of aquatic life. The presence of oxygen in water is a good sign. Reduction of dissolved oxygen in water supplies can promote the microbial reduction of nitrate to nitrite and sulphate to sulphide, which can also cause an increase in the concentration of ferrous iron

Principle:-

Dissolved oxygen is measured by titrimetric or electrometric method. A water sample is taken in a “dissolved oxygen” bottle. A bottle with specially-designed cap (or specially-designed mouth and glass stopper, often sold as “BOD bottle”), which permit enclosure of liquids without contact with air. Chemical reagents added in excess interact with oxygen to form a product, and another chemical (the “titrant”) is used quantitatively to “neutralize” that product. The amount of titrant needed is proportional to oxygen concentration and is translated to mg/l. Among all the Winkler method is the best known titrimetric method for dissolved oxygen. this method and its modifications are widely applied, both in the field as well as in lab.

DO can be determine by two methods titrimetric method or electrometric method. Titrimetric method is centred on the oxidizing property of DO where as the electrometric method is centred on the rate of
diffusion of molecular oxygen across a membrane. There are different types of titrimetric method which are:
(a) Winkler Method , (b) Azide Modification , (c) Alum Flocculation Modification , (d) Permanganate Modification

**The Titrimetric principle:** It is the most accurate method to find out DO. Divalent Manganese salt in solution is precipitated by strong alkali to divalent manganese hydroxide. 

\[
MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4
\]

Addition of Potassium iodide or Potassium hydroxide is added to create a pinkish brown precipitate. In the alkaline solution, dissolved oxygen present in the sample rapidly oxidized to form trivalent or higher valency hydroxide.

\[
2 Mn(OH)_2 + O_2 \rightarrow 2 MnO(OH)_2
\]

MnO(OH)_2 appears as a brown precipitate. There is some confusion about whether the oxidised manganese is tetravalent or trivalent. Some sources claim that Mn(OH)_3 is the brown precipitate, but hydrated MnO_2 may also give the brown colour. Iodide ions are added and acidified, which reduces tetravalent hydroxides back to their stable divalent state thereby liberating equivalent amount of iodine.

\[
Mn(OH)_2 + 2KI + H_2O \rightarrow Mn(OH)_2 + I_2 + 2 KOH
\]

Thiosulphate solution is used, with a starch indicator, to titrate the iodine.

\[
I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I\]

This iodine is equivalent to dissolved oxygen present in the sample.

**Apparatus Required :-** Burette, Burette stand, 300 mL glass stoppered BOD bottles, 500 mL conical flask, Pipettes with elongated tips, Pipette bulb, 250 mL graduated cylinders, Wash bottle.

**Chemicals Required:** Manganous Sulphate Solution, Alkaline Iodide Azide Solution, Con H_2SO_4, Starch indicator Solution, Sodium Thiosulphate, Potassium Hydroxide, Potassium Iodide, Sodium Azide and distilled water.

**Preparation Of Reagents:-**

1. **Manganous Sulphate Solution:-**
   - Take 480g of MnSO_4.4H_2O or 400g MnSO_4.2H_2O or 364 g MnSO_4.H_2O
   - Dissolve it in distilled water and then filter the solution and make the volume up to 1000 ml.

2. **Alkaline Iodide Azide Solution:-**
Dissolve either 500 g of NaOH or 700 g of KOH and 135 g of NaI or 150 g of KI.

To prepare this reagent, take 700 g of Potassium hydroxide and add 150 g of potassium iodide and dissolve it in freshly boiled and cooled water, and make up to 1000 ml.

Dissolve 10 g of Sodium Azide in 40 mL of distilled water and add this with constant stirring to the cool alkaline iodide solution prepared.

3) Sodium Thiosulphate Solution:

- Take 25 g of sodium thiosulphate and dissolve it in boiled distilled water and make the volume up to 1000 ml.
- Add 1 g of Sodium Hydroxide to preserve it.

4) Starch Indicator:

- Weigh 2 g of starch and dissolve in 100 mL of hot distilled water.
- Add 0.2 g of salicylic acid as preservative, if you are going to preserve it.

Calculation of Dissolved Oxygen:

\[
\text{Dissolved Oxygen} = \frac{\text{Volume of Sodium Thiosulphate} \times 0.2 \times 1000}{\text{Volume of sample taken}}
\]

PROEDURE CHART:

1. Fill the water sample in 300 ml glass stoppered BOD bottle up to the rim by avoiding bubbling.
2. Add 2mL of manganese sulfate to the BOD bottle by inserting the calibrated pipette inside the water sample.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner. Pipette should be dipped inside the water sample. Avoid bubbling.
4. If oxygen is present, a brownish-orange cloud of precipitate will appear. Allow the precipitation settle down to react completely with oxygen.
(b) BIOLOGICAL OXYGEN DEMAND:-

**Introduction:**

The Biological Oxygen Demand (BOD) determination is the chemical procedure for measurement the total amount of oxygen required by aerobic organisms present in water bodies. BOD of water is defined as the amount of oxygen required for the biological decomposition of organic matters dissolved in it. Generally, the time is taken as 5 days and the temperature is 20°C.

**Principle:**

The water sample is filled in glass stoppered airtight BOD bottles and incubated at specific temperature about 20°C for 5 days. The DO of water sample is measured before and after 5 days.
of incubation. The initial DO is determined soon after the dilution is made; all oxygen uptakes occurring after this measurement is included in the BOD measurement.

**Apparatus Required:**- BOD Incubator, Burette & Burette stand, 300 ml glass stopper BOD bottles, 500 ml conical flask, Pipettes with elongated tips, Pipette bulb, 250 ml graduated cylinders, wash bottle.

**Chemicals Required:**- Manganous sulphate, Potassium hydroxide, Potassium iodide, Sodium azide, concentrated sulphuric acid, Starch indicator, Sodium thiosulphate, Distilled Water.

**Sample Handling And Preservation:**-
No need to preserve sample practically because biological activity will continue even after collecting a sample, changes may occur during handling and storage. If analysis is not going to be done within two hours of collection, cool storage is not necessary. If it cannot be started within two hours of sample collection to reduce the change in sample, keep all samples at 4° C. Do not allow samples to freeze. Do not open sample bottle before analysis. Begin analysis within six hours of sample collection.

**Preparation Of Reagent:**-

1. **Manganous Sulphate Solution:**-
   
   As prepared in DO test

2. **Alkaline Iodide Azide Solution:**-
   
   To prepare this solution mix three different chemicals as per DO test.

3. **Sodium Thiosuphate Stock Solution:**-
   
   As prepared in DO test

4. **Starch Indicator:**-
   
   As prepared in DO test

5. **Calcium Chloride Solution:**-
   
   - Weigh exactly 27.5 gm of anhydrous calcium chloride and dissolve it in distilled water.
   - Transfer it to 100 ml flask and make final volume 100 ml using distilled water.
   - Add 5 ml phosphate buffer solution.

6. **Magnesium Sulphate solution:**-
   
   - Weigh accurately 22.5 gm of Magnesium Sulphate and dissolve it in distilled water.
   - Transfer it to 100 ml flask and make final volume 100 ml adding distilled water.

7. **Ferric Chloride solution:**-
➢ Take exact 0.15 gm of ferric chloride and dissolve it in distilled water.
➢ Transfer it to 100 ml flask and make final volume 100 ml adding distilled water.

(8) Phosphate Buffer solution:-
➢ Weigh exactly 8.5 gm of Potassium Di Hydrogen Phospbate (KH$_2$PO$_4$) and dissolve it in distilled water.
➢ Then after add 21.75 gm of Di Potassium Hydrogen Phosphate (K$_2$HPO$_4$) and dissolve it in distilled water.
➢ Then to the same beaker add 33.4 gm of Di Sodium Hydrogen Phosphate (Na$_2$HPO$_4$. 2H$_2$O) and 1.7 gm of (NH$_4$Cl) and dissolve it.
➢ Transfer the content to 1000 ml flask and make final volume 1000 ml by adding distilled water.

(9) Dilution water:- High quality organic free water must be used as dilution water. The required volume of water is aerated by a supply of clean compressed air for 12 hours. Allow it for stabilization by incubating at 20°C for 4 hours.

PROCEDURE CHART:-

1. Add 5 ml of Calcium chloride solution and 5 ml of Magnesium Sulphate solution in the beaker containing 5L dilution water
2. Add 5 ml of Ferric chloride solution and 5 ml of Phosphate buffer solution in the beaker containing 5L dilution water
3. Now take 300 ml BOD bottles as per requirement.
4. Add 30 ml of water sample and remaining 270 ml filled with dilution water.
5. Take 2 BOD bottles and filled with dilution water alone for blank reading.
6. After that immediately put the glass stopper over the BOD bottles and give number to the bottles for identification
(c) CHEMICAL OXYGEN DEMAND:-

The chemical oxygen demand (COD) is defined as the amount of organic compound present in water. It is the measurement of the amount of oxygen in water consumed for chemical oxidation of organic matter in water or waste water sample. It is expressed in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution. This method covers the determination of COD in ground and surface waters, domestic and industrial wastewaters. The relevant range is 3-900 mg/L.

**Principle:-**

Potassium dichromate (K$_2$Cr$_2$O$_7$) oxidised the various organic matters present in water sample in the present of sulphuric acid (H$_2$SO$_4$), silver sulphate(AgSO$_4$) and mercury sulphate(HgSO$_4$) to produce CO$_2$ and H$_2$O. The sample is refluxed with potassium dichromate (K2Cr2O7) in the medium of sulphuric acid and the surplus potassium dichromate (K2Cr2O7) is determined by titration against ferrous ammonium sulphate, using ferroin indicator. The dichromate consumed by the sample is equivalent to the amount of O2 required to oxidize the organic matter.

**Apparatus Required:-** COD Digester, Burette & Burette stand, COD Vials with stand, 250 mL conical flask (Erlenmeyer flask), Pipettes, Pipette bulb, Tissue papers, Wash Bottle.

**Chemicals Required:-** Potassium dichromate, Sulphuric acid, ferrous ammonium sulphate, Silver sulphate, Mercury sulphate, Ferroin indicator, distilled water.

**Sample Handling And Preservation:-**

Samples should be collected in glass bottles. If glass bottles are not convenient for collection then plastic bottles can be used but they must be free from any type of contamination. Samples
should be preserved with sulphuric acid to a pH < 2 and maintained at 40°C until analysis. Freezing of sample is strictly not allowed.

**Preparation Of Reagents:-**

(1) **Standard Potassium Dichromate Reagent (Digestion solution):**

- Weigh 4.913 g of potassium dichromate and transfer it to beaker.
- Take 33.3 g of mercuric sulphate and add to the same beaker.
- Measure exact 167 mL of concentrated sulphuric acid. Dissolve the contents and cool to room temperature. If not dissolved keep it overnight.
- Make up the final volume up to 1000 mL by adding distilled water. Which is to be used for digestion.

(2) **Sulphuric Acid Reagent:**

- Take 5.5 g silver sulphate crystals to a dry clean 1000 mL beaker.
- Then carefully add about 500 mL of concentrated sulphuric acid and allow it to stand for 24 hours to dissolve silver sulphate crystals completely.

(3) **Standard Ferrous Ammonium Sulphate Solution:**

- Take exact 39.2 g of ferrous ammonium sulphate crystals and dissolve it in distilled water.
- Then carefully transfer the contents to the 1000 mL standard flask and make the volume up to 1000 mL by adding distilled water.

**Calculation for COD:**

\[
\text{Chemical Oxygen Demand} = \frac{(A-B) \times N \times 8 \times 1000}{\text{Volume of sample taken}}
\]

Where:
A = Volume of Ferrous Ammonium Sulphate for blank.
B = Volume of Ferrous Ammonium Sulphate for sample.
N = Normality of Ferrous Ammonium Sulphate.

**PROCEDURE CHART:**

1. Add 2.5 mL of the sample to each COD vials and one COD vial is for blank, in which add distilled water.
Carbon dioxide is a normal component of natural waters. In polluted water it is formed by the biological oxidation of organic matter. Carbon Dioxide is present in water in the form of a dissolved gas. Free CO$_2$ in water accumulates due to microbial activities and respiration of organisms. Free CO$_2$ can be determined by the titrimetric method using strong alkali to pH 8.3. Surface waters usually contain less than 10 mg/L free carbon dioxide, while some ground waters it may easily go above that concentration up to 30 to 50 mg/L. Over the normal temperature range (0-30$^\circ$C) the solubility is about 200 times than of oxygen. Calcium and magnesium combine with carbon dioxide to form carbonates and bicarbonates. Aquatic plant life depends upon carbon dioxide and bicarbonates in water for growth. Microscopic
aquatic plant life found submerged in the water, phytoplankton, free floating plants as well as large rooted plants, use carbon dioxide in the photosynthesis necessary to prepare food for plant materials such as starches, sugars, oils, proteins. The carbon in all these materials comes from the carbon dioxide in water. The concentration of carbon dioxide rises when the oxygen concentration in water is reduced. The rise in CO$_2$ causes problems and make more difficult for fish and other aquatic animals.

**Principle:-**
Free CO$_2$ reacts with sodium carbonates to form sodium bicarbonate or with sodium hydroxide to form sodium carbonate. Pink color development indicated that the reaction is completed, pink color developed due to phenolphthalein indicator at the correspondence pH of 8.3.

**Sample Handling And Preservation:-**
Water sample should be collected more carefully, although some loss in free CO$_2$ can be expected in storage. This occurs when the gas is present in large amounts. Sometimes a sample may show increase in CO$_2$ content on standing. So it is advisable to determine free CO$_2$ at the point of collection. Where field determination is not suitable, fill the bottle completely for laboratory testing. Keep the sample, until examination, at a temperature lower than that at which the water was collected. Make the laboratory test as soon as possible to diminish the effect of CO2 changes.

**Chemicals Required:-** Sodium Hydroxide, Phenolphathalein, distilled water

**Preparation Of Reagents:-**

1. **Sodium Hydroxide Solution (0.02 N) :-**
   - Take 1000 mL standard measuring flask and fill 3/4th of it with distilled water.
   - Accurately measure 20 mL of 1N Sulphuric acid solution using a pipette and transfer to 1000 mL standard flask containing the distilled water.
   - Make the final volume up to 1000 mL using distilled water.

2. **Phenolphathalein Indicator:-**
   - Take 1 gm. of phenolphthalein and dissolve it in 100 mL of 95% ethyl alcohol.

**PROCEDURE CHART:-**

1. Take 20 mL water sample in conical flask. 
2. Add few drops of methyl orange indicator in the conical flask. The colour changes to pink.
Chapter III 

Materials and Methods

Calculation for free CO\(_2\):-

\[
\text{Volume of NaOH x N x 50 x 1000} \\
\text{FREE CO}_2 = \frac{\text{Volume of NaOH x N x 50 x 1000}}{\text{Volume of water sample}}
\]

Table 6 :- Methods Used For Water Analysis:

<table>
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<tr>
<th>Sr.NO</th>
<th>PAREMETERS</th>
<th>METHODS USED</th>
</tr>
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<tr>
<td>1</td>
<td>pH</td>
<td>pH Meter (Equip Trohics)</td>
</tr>
<tr>
<td>2</td>
<td>EC</td>
<td>Conductivity method (Equip Trohics) Model no. EQ-660</td>
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<tr>
<td>3</td>
<td>Turbidity</td>
<td>Turbidity meter, Model No – LT-33</td>
</tr>
<tr>
<td>4</td>
<td>TDS</td>
<td>Conductivity method</td>
</tr>
<tr>
<td>5</td>
<td>Ca(^{+2})</td>
<td>EDTA Titrimetric method</td>
</tr>
<tr>
<td>6</td>
<td>Ca(^{+2}) + Mg(^{+2})</td>
<td>EDTA Titrimetric method</td>
</tr>
<tr>
<td>7</td>
<td>Na(^{+})</td>
<td>Flame photo metrically</td>
</tr>
<tr>
<td>8</td>
<td>K(^{+})</td>
<td>Flame photo metrically</td>
</tr>
<tr>
<td>9</td>
<td>PO(_4)(^{-1})</td>
<td>Digital Spectrophotometrically, Model no: EQ-825</td>
</tr>
<tr>
<td>10</td>
<td>Cl(^{-})</td>
<td>Argentometric method</td>
</tr>
<tr>
<td>11</td>
<td>CO(_3)(^{-2}) + HCO(_3)(^{-2})</td>
<td>Neutralization method (Titrimetric against H(_2)SO(_4))</td>
</tr>
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<td>12</td>
<td>Free CO(_2)</td>
<td>NaOH Titrimetric method</td>
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<td>13</td>
<td>Dissolved oxygen DO)</td>
<td>Winkler method</td>
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<td>14</td>
<td>BOD</td>
<td>5 Day BOD test</td>
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<tr>
<td>15</td>
<td>COD</td>
<td>Open reflux method</td>
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</tbody>
</table>