CHAPTER III

EXPERIMENTAL METHODS
The procedures adopted for the estimation of various physical and chemical parameters as described in the standard methods\textsuperscript{11-14}.

1. **COLLECTION OF SAMPLES:**

   **Well Water samples:**

   The well and bore-well water samples were collected in clean polythene bottles (2 or 5 liters capacity) after rinsing the bottle with the sample. For the estimation of dissolved oxygen, BOD bottles have been used as recommended and the dissolved oxygen was fixed at the site of collection.

2. **METHODS OF PRESERVATION OF SAMPLES:**

   In Table-3, the methods of preservation of sample adopted along with the maximum time taken for the estimation of various chemical parameters is described.

3. **REAGENTS:**

   All the chemicals, reagents used in this work were of analytical grade of E. Merck, India, Glaxo India, and S.D. Chemicals India.

   Distilled water was generally used for the preparation of Solutions.
4. **PREPARATION OF REAGENTS AND SOLUTIONS:**

**pH:**

Potassium Hydrogen phthalate (0.05M): 1.02gms of potassium hydrogen phthalate was dissolved in distilled water and diluted to 100mL.

i. **Borax Buffer (0.01M):** 0.381gms of sodium borate was dissolved in distilled water and diluted to 100mL.

**Electrical Conductivity (E.C):**

Standard Potassium Chloride solution (0.01M): 0.7456gms of potassium chloride was dissolved in conductivity water (deionized water) and diluted to 1000mL.

**Total Hardness, Calcium and Magnesium:**

i. **Standard Magnesium sulphate solution:** 0.6161gms (0.01M) of Magnesium sulphate was dissolved in distilled water and diluted to 250mL.

ii. **Ethylene diamine tetra acetic acid solution (0.01M):** 3.723gms of disodium ethylene diamine tetra acetic acid dehydrate was dissolved in distilled water and diluted to 1000mL. This was standardized against standard Magnesium sulphate solution just before the experiment was performed.
iii. **Buffer Solution (pH=10):** 16.9gms of ammonium chloride was dissolved in 143mL of concentrated ammonium hydroxide and then diluted to 250mL with distilled water.

iv. **Eriochrome Black-T indicator solution:** The indicator solution was prepared by dissolving 0.5gms of Eriochrome Black-T in 100mL of triethanolamine.

v. **Murexide Indicator:** 200mg of the dye were mixed with 100gm of sodium chloride.

vi. **Sodium hydroxide solution:** 4gm of sodium hydroxide was dissolved in 100mL distilled water.

vii. **Starch Indicator solution:** 1gm of starch powder was dissolved in 100mL of hot distilled water.

**Chloride:**

i. **Standard Potassium Chloride Solution:** 0.932gms of potassium chloride was dissolved and diluted to 500mL with distilled water.

ii. **Silver Nitrate Solution:** 4.2468gms of silver nitrate was dissolved with distilled water and diluted to 1000mL. This was standardized against standard chloride solution.
iii. **Potassium chromate indicator solution**: 5gms of Potassium Chromate was dissolved in distilled water and diluted to 100mL.

**Dissolved Oxygen:**

i. **Manganous sulphate solution**: 50gms of manganous sulphate was dissolved in distilled water and diluted to 100mL.

ii. **Alkali Iodide (NaOH + KI)**: 50gms of Sodium hydroxide 15mg of potassium iodide were dissolved in distilled water and diluted to 100mL.

iii. **Sodium thiosulphate solution**: 3.1025gms of sodium thiosulphate and 0.4gm of sodium hydroxide dissolved in distilled water and made up to 1000mL.

iv. **Standard Potassium dichromate solution**: 0.4903gm potassium dichromate was dissolved in distilled water and diluted to 100mL.

**Biochemical Oxygen demand:**

i. **Phosphate buffer**: 0.85gms of potassium dihydrogen phosphate, 2.18gms of dipotassium monohydrogen phosphate, 3.34gms of disodium hydrogen phosphate and 0.17gm of ammonium chloride were
dissolved in 70mL distilled water and diluted to 100mL. The pH of the solution will be 7.2.

ii. **Calcium chloride solution**: 2.75gm of calcium chloride was dissolved in 100mL.

iii. **Magnesium sulphate solution**: 8.25gm of anhydrous Magnesium sulphate was dissolved in 100mL distilled water.

iv. **Ferric chloride solution**: 025gm of Ferric chloride was dissolved in 1000mL distilled water.

5. **INSTRUMENTS:**

**pH Meter:**
Systronics digital pH meter type 335 was used to measure the pH of the samples.

**Conductivity meter:**
Systronics digital direct reading conductivity meter type 304 was used to measure the electrical conductivity of the samples.

**BOD Incubator (KBI 10S):**
Kumar make with digital temperature controlled BOD incubator was used for BOD determination.

6. **GENERAL PROCEDURES FOR THE DETERMINATION OF VARIOUS CHEMICAL PARAMETERS IN THE SAMPLES:**
**pH:**

The glass electrode was standardized against buffer solutions of known pH values of (pH 4 and pH 9.2) at 25°C. About 100mL of sample was taken in a beaker; the electrode was kept immersed in the sample and measured the pH.

**Electrical Conductivity:**

The conductivity meter was standardized against standard potassium chloride solution at 25°C. About 100mL of sample was taken in beaker, the conductivity cell was kept immersed in the sample and measured the electrical conductivity.

**Total Dissolved Solids:**

100mL of the filtered sample was taken in a previously dried and weighed beaker and evaporated to dryness and it was weighted. The amount of total dissolved solids (TDS) was calculated using the formula.

\[
TDS \ (mg/L) = \frac{(A-B) \times 1000}{V}
\]

Where, 

\[A = \text{Weight of the residue and beaker (mg)}\]

\[B = \text{Weight of the beaker (mg)}\]

\[V = \text{Volume of the sample taken}\]
**Total Suspended Solids (TSS):**

250mL of the sample was taken exactly in a volumetric flask and allowed to filter through a dried and weighed gooch crucible containing an asbestos mat. The suspended solids retained in the crucible were washed with distilled water to remove chloride. The crucible was finally dried, cooled in desiccators and weighted. The increase in the weight of the crucible was noted. The amount of Total suspended Solids (TSS) was calculated using the formula.

\[
TSS \text{ (mg/L)} = \frac{(A-B) \times 1000}{V}
\]

Where,

- \(A\) = Increase in weight of the crucible (gms)
- \(B\) = Weight of the empty crucible (gms)
- \(V\) = Volume of the sample taken

**TOTAL HARDNESS:**

**i) Standardization of EDTA Solution:**

20mL of the standard magnesium sulphate solution was taken in a conical flask to which ammonium chloride-ammonium hydroxide buffer solution was added, such that the pH of the final solution was added, such that the pH of the final solution was 10 and 1-2 drops of Eriochrome Black – T indicator solution was added and the solution was thoroughly mixed and titrated using EDTA solution till the color changes from wine red to blue. From the volume of EDTA solution consumed and from the concentration of
standard Magnesium sulphate solution, the strength of EDTA solution was calculated.

**ii) Determination of Total Hardness:**

20mL of the filtered sample was taken in a conical flask to which ammonium chloride-ammonium hydroxide buffer solution was added such that the pH of the final solution was 10 followed by the addition of 1 - 2 drops of Eriochrome Black – T indicator solution. The solution was titrated with standard EDTA solution slowly with continuous stirring, till the color changes from wine red to blue. The total hardness is expressed in terms of milligrams per liter of calcium carbonate equivalent and calculated using the following formula:

Total Hardness (mg (CaCO$_3$) / L) = $\frac{A \times B \times 1000}{V}$

Where,

- A = Volume of EDTA consumed for the sample (mL)
- B = mg of CaCO$_3$ equivalent to 1mL of EDTA
- V = Volume of sample taken (mL)

**Calcium and magnesium:**

20 ml of filtered sample was taken in a conical flask to which 20 ml of hydroxide buffer solution was added such that pH of the final solution was between 12 to13. 0.1 to 0.2 gm to 0.2gm of muroxide indicator was added and the solution was thoroughly mixed and titrated with standard EDTA solution with continuous
stirring till the colour changes from pink to purple at the end point. Calcium present in the sample from the above titration was calculated as follows

Calcium (mg. Ca/lt) = \( \frac{A \times 0.1008 \times 1000}{V} \)

Where, \( A = \) Volume of EDTA consumed for the sample (ml) 
\( V = \) Volume for sample taken (ml)

Magnesium (mg.Mg/lt) = \( \frac{(Y-X) \times 100.8}{V} \)

Where, \( Y = \) Volume of EDTA consumed for the sample for hardness determination (ml) 
\( X = \) Volume of EDTA consumed for co determination 
\( B = \) Volume of sample taken (ml)

**CHLORIDE** (Argentometric method):

i) **Standardization of Silver nitrate Solution:**

10 ml of standard sodium Chloride solution was taken in a conical flask and 40 ml of distilled water followed by 1 ml of potassium chromate solution were added. The solution was titrated against silver nitrate solution till it changes from yellow to red. The same procedure was adopted for the blank also. From the volume of silver nitrate solution consumed and from the concentration of standard sodium chloride solution the strength of the silver nitrate solution was calculated.
ii) **Determination of Chloride:**

50 ml of sample was taken in a conical flask. 1 ml of potassium chromate solution was added and titrated with standard silver nitrate solution till it changes from yellow to red. The concentration of chloride was calculated using the following formula.

\[
\text{Chloride (mg/lt)} = \frac{(A-B) \times 35.45 \times 1000}{V}
\]

Where,

A = Volume of silver nitrate solution consumed for sample (ml)

B = Volume of silver nitrate solution consumed for blank (ml)

V = Volume of sample taken (ml)

**DISSOLVED OXYGEN:**

i) **Standardization of sodium thiosulphate solution:**

10.0 ml of standard potassium dichromate solution was taken in a conical flask. 4 ml of hydrochloric acid, 10 ml of 10% potassium iodide followed by 30 ml of distilled water were added. The liberated iodine was titrated with sodium thiosulphate solution with a constant stirring of the solution. The titration was continued till the solution attains yellowish green colour and then 2 ml of freshly prepared starch solution was added. The colour of the solution changes to blue. The titration was continued by the drop wise addition of sodium thiosulphate until the blue colour changes to light green. Blank titration was also carried out.
ii) Determination of Dissolved Oxygen:

As mentioned previously, the sample solution was collected carefully in 300 ml BOD bottle. To this, 2ml of manganese sulphate solution and 2 ml of alkali iodide reagent were added. The pipette tips were held just below the liquid surface while adding the reagents. The sample bottles were stoppered carefully to exclude any air bubbles. Then the contents of the bottle were mixed by inverting the bottle few times mixed the contents of the bottle. Concentrated sulphuric acid in 1 ml fractions was added till manganous hydroxide flocks were dissolved. From this solution, and aliquot of 50 ml was taken and titrated with 0.025 M standard sodium thiosulphate solution to a pale straw colour. Then few drops of freshly prepared starch solution was added and the titration was continued to the first disappearance of the blue colour. The dissolved oxygen present in the solution was calculated as follows

\[
\text{Dissolved Oxygen (mg/lt) } = \frac{A \times N \times 0.008 \times 10^6}{V}
\]

Where,

\( A = \) Volume of sodium thiosulphate solution consumed for the sample (ml)

\( N = \) Normality of sodium thiosulphate solution

\( V = \) Volume of sample taken (ml)
**BIO – CHEMICAL OXYGEN DEMAND**

i) **Preparation of the dilution water:**

Required quantity of water was taken in 1000ml volumetric flask to which 1 ml of each of phosphate buffer, magnesium sulphate, Calcium chloride and ferric chloride solutions were added. Whenever necessary, required quantities of seeding in the form of domestic sewage water was added to supply sufficient population of micro organisms. This dilution water was so prepared that the dissolved oxygen uptake in 5 days was not more than 0.2 mg/lt. and stored.

ii) **Determination of Bio-chemical Oxygen Demand:**

The sample collected in a BOD bottle was cooled in an ice-bath was brought back to 20°C. Several dilutions of the sample in duplicate were made depending on the nature of sample. The dissolved oxygen in one of the bottles was measured as described previously. The BOD bottles after proper seeding are incubated in BOD incubator for 5 days at 20 °C. The dissolved oxygen in the incubated sample was measured. The same procedure was repeated for dilution water, which was used as a blank. The BOD was calculated by using the following formula. When dilution water is not seeded.

\[
\text{BOD, 5 day, 20 °C (mg/lt) = } [(D_0-D_1)-(C_0-C_1)]P
\]

Where
$D_0 =$ Dissolved oxygen in the sample immediately after preparation (mg/lt.)

$D_1 =$ Dissolved oxygen in the sample after 5 days incubation at 20 °C (mg/lt.)

$C_0 =$ Dissolved oxygen in the blank immediately after preparation (mg/lt.)

$C_1 =$ Dissolved oxygen in the blank after 5 days incubation at 20 C (mg/lt.)

$P =$ Decimal fraction of sample used.

This is to inform that BOD values were not reproducible and there was a possibility of high percentage error in these values, due to frequent failure of electric power.

**ALKALINITY:**

Alkalinity of water is due to the presence of carbonate, bicarbonate and hydroxide ions.

**Determination of Alkalinity:**

100 ml of the water sample was taken in a conical flask. 2 drops of phenolphthalein indicator was added. It was then titrated with 0.02N H$_2$SO$_4$ till it becomes colourless. Then 2 drops of methyl orange indicator was added and the titration was continued till the colour changes from yellow to pink.
Calculations:

Phenolphthalein alkalinity (as CaCO₃) mg/lt. =

\[
\frac{V1 \times \text{Normality of } H_2SO_4 \times 1000 \times 50}{\text{volume of sample taken}}
\]

Where,

V1 = Volume of the acid consumed for phenolphthalein end point

Total alkalinity (as CaCO₃) mg/lt. =

\[
\frac{V2 \times \text{Normality of } H_2SO_4 \times 1000 \times 50}{\text{volume of sample taken}}
\]

V2 = Volume of the acid consumed for methyl orange end point.