Section I

Water Analysis

Chapter 1

Chemical Analysis of Water

1.1 pH

The pH gives a scale of available hydrogen ion concentration in water. If free H^+ ions are more than OH^- ions, the water will be acidic, or otherwise alkaline. The most chemically pure water at 22℃ is partly dissociated into H^+ and OH^- ions. This quantity is exactly 10^{-14} g molecules of dissociated H^+ and OH^- ions. Therefore, the H^+ ions are 10^{-7}. This value is expressed in terms of negative logarithm of the total H^+ ion concentration. Hence, pH = 7 indicates neutral water, below this scale is acidic and above alkaline.

Fig. 1. *Laboratory and field pH meter.*

(1) Measurement Procedure

pH of water can be accurately measured using pH meter of different makes. Most of the pH meters come with following accessories/chemicals:

1. One digital pH meter with electrode.
2. Two sets of buffer solutions, mostly of pH 4.0 and 9.2.

BOX 1

However, buffers of these two ranges (4.0 and 9.2) can be prepared from different buffer solutions. Some of these are:

1. Phthalate buffer: A solution of 10.2 g Potassium hydrogenphthalate + 1L distilled water gives pH of 4.0 (±0.01).
2. Phosphate buffer: A solution of 3.4 g of KH_2PO_4+4.45 g of $NH_2HPO_4.2H_2O$ + 1L distilled water gives a pH of 6.9 (±0.05).
3. Borax buffer: A solution of 3.81 g of $Na_2B_4O_7.10H_2O$ + 1L distilled water gives a pH of 9.3 (±0.12).

Operation procedures for different pH meters are different. However, following procedure is described for most usual pH meters.

1. Switch on the pH meter and allow some time for stabilization.
2. Wash the electrode with distilled water and connect to electrode holder of the pH meter.
3. Dip the electrode in buffer solution of pH 4.0 and move the temperature knob to specified buffer temperature. Adjust the set buffer knob until it reads 4.0.
4. Turn to selector switch 0.
5. Wash the electrode with distilled water and dip in buffer pH solution 9.2.
6. Adjust the set buffer knob until it reads 9.2.
7. Turn selector switch to 0.
8. Wash the electrode with distilled water.
9. Read the pH of the sample by dipping the electrode into the sample. Fix the temperature knob to the temperature of the sample.
10. Wait for some time and note the reading.

(2) Caution

Prolonged dipping of electrode into the sample may cause variation in reading. Now a day, portable pH meters are available which are handy and does not require any buffer setting before use.

1.2 Electrical Conductivity

Electrical conductivity is a measurement required for brackish or sea water. In case of pollution studies, this parameter is required for fresh water.

(1) Principle

Electrical conductivity focuses on dissolved salts or their ions in water which are good conductors of an electrical current. It is measured by a probe that applies voltage between two electrodes, spaced a known distance apart, and records the drop in voltage. This drop reflects the resistance of the water, which is then converted to conductivity. Thus, conductivity is the inverse of resistance and is measured in the amount of conductance over a certain distance. The conductivity units are called "mhos" - the inverse of "ohms" used in resistance. For most natural waters, the units of mhos/cm are too large, so conductivity is reported as micro-mhos/cm where 10^6 micromhos is equal to one mho. Sometimes the units are expressed as microSiemens and 1 microS is equal 1 micromhos/cm.

(2) Requirements

Electrical Conductivity meter.

(3) Procedure

Most of the Electrical Conductivity meter comes with a temperature calibration knob and two probes (electrodes) for conductivity measurement. Electrical conductivity varies at different temperatures. Therefore, it is necessary to set the temperature of Electrical Conductivity meter as close as possible to the ambient temperature of the sampling station. The probes are then dipped to water for some time (15-20 minutes). Keeping probes under water for a longer time will give different results since salts or ions start depositing on the probes.

Electrical Conductivity also represents salinity of water. However, a g/l conversion can be made if the ratio of salts present in water sample is known.

1.3 Total Alkalinity

(1) Principle

The amount of acid required to titrate the bases in water are a measure of the alkalinity of water. Water contains a number of bases, including carbonates, bicarbonates, hydroxides, silicates, phosphates, ammonia and various organic compounds occur in water. However, bicarbonates (HCO_3^-), carbonates (CO_3^-) and hydroxides (OH^-) are considered as the predominant bases in natural waters. Since water with pH value of about 4.5 and above may contain bicarbonate, water sample that turn yellow upon the addition of methyl orange indicates alkalinity.

(2) Why $CaCO_3$ Alkalinity

Alkaline earth carbonates such as calcite or dolomite are the principal sources of bases in water, so alkalinity has been traditionally expressed as mg/L of $CaCO_3$. The standard H_2SO_4 used for alkalinity titration is often of such strengths that 1 ml is exactly equal to 1mg of $CaCO_3$.

(3) How to Express Alkalinity

Results of alkalinity titration is expressed as total alkalinity or as individual components of alkalinity, i.e. hydroxide, carbonate and bicarbonate alkalinity.

(4) Phenolphthalein and Methyl Orange Alkalinity

When, in addition of phenolphthalein indicator, water sample turns to pink, (pH above 8.4), they contain measurable carbonate ions. In that case, alkalinity titration is carried out in two steps.

(1) The sample is first titrated to the phenolphthalein end point which turns all CO_3^{2-} to HCO_3^-

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^-$$

(2) The sample is then titrated with H_2SO_4 until all of the HCO_3^- is converted to

carbon dioxide and water at the methyl orange end point.

$$HCO_3^- + H^+ \leftrightarrow H_2O + CO_2$$

(5) Reagents

Phenolphthalein indicator: Dissolve 0.5gm of phenolphthalein in 50ml of 95 % ethyl alcohol and add 50ml distilled water.

Methyl orange indicator: Dissolve 0.05gm of methyl orange in 100ml of distilled water.

Standard Sodium Carbonate Na_2CO_3, 0.0200N: Dissolve 1.0600gm of anhydrous Na_2CO_3 and dilute to 1000ml in CO_2 free distilled water. Boil distilled water for 10 to 15 minutes to expel CO_2 and cool before using. The Na_2CO_3 must be used within a few hours of preparation.

Standard H_2SO_4 titrant, 0.0200N: Prepare H_2SO_4 stock solution of approximately 0.1 N by diluting 2.8ml of conc. H_2SO_4 to 1000ml with CO_2 free distilled water. This solution is approximately 0.02N, but it must be carefully standardized to determine its exact normality. To standardize, pipet 10.00ml of 0.02N Na_2CO_3 into a 250ml beaker. Add 90ml of CO_2 free distilled water and 4 to 8 drops of methyl orange indicator solution. Select a number of drops of methyl orange which allows easy end point detection and use this number of drops in all subsequent titrations. Titrate over a white surface to the methyl orange end point with standard H_2SO_4. At the end point, one drop of acid will change the colour of methyl orange from yellow to faint orange. Calculate the normality of the sulphuric acid from the following equation:

$$NV = N'NV'$$

Where,

N= Normality of the standard;

V= Milliliter of the standard used in titration;

N' = Normality of the solution being standardized;

V ′= Volume of the solution in milliliters'.

(6) Procedure for Phenolphthalein Alkalinity

- Measure 100ml of water sample into a 250ml beaker.
- Add 2 drops of phenolphthalein indicator solution.

- If the sample turns pink, it contains phenolphthalein alkalinity. Titrate with standard H_2SO_4 solution until one drop of acid causes the pink colour of the sample to disappear.
- This sample can be saved for methyl orange alkalinity.
- Calculate the phenolphthalein alkalinity by following equation:

$$\text{Phenolphthalein alkalinity} = \frac{(\text{ml of titrant use})(\text{N})(50)(1000)}{\text{Sample volume in ml}}$$

(7) Total Alkalinity

- Take 100ml of fresh sample and add 4 to 8 drops of methyl orange indicator solution to it.
- Titrate with standard H_2SO_4 until the colour of the solution changes from yellow to faint orange.

(8) Measuring Total Alkalinity on Sample from Phenolphthalein Alkalinity

- Titration can be carried out on the sample used to measure phenolphthalein alkalinity.
- Add 4 to 8 drops of methyl orange indicator.
- Now titrate against the titrant used (in ml) in total alkalinity before calculating the total alkalinity of this sample.

$$\text{Total alkalinity} = \frac{(\text{ml of titrant used})(\text{N})(50)(1000)}{\text{Sample volume in ml}}$$

1.4 Total Hardness

(1) Introduction

The concentration of calcium plus magnesium ions expressed as equivalent $CaCO_3$ has traditionally been taken as a measure of total hardness. Other divalent metals also contribute to hardness, but their concentrations are negligible in natural waters.

(2) Principle

Calcium and magnesium ions are titrated with the complexing agent ethylene diamine tetra acetic acid (EDTA) to form the stable complexes CaEDTA and MgEDTA. The end point of the titration is signaled with a second complexing

agent, Erichromic Black -T.

If a small quantity of Erichromic Black-T is added to a water sample buffered at pH 10, it will form a soluble wine red complex with some of the calcium and magnesium ions. In the titration, the EDTA will first complex all of the free Ca^{2+} and Mg^{2+}, and then Calcium and Magnesium will dissociate from their complexes with Erichromic Black-T to form more stable complexes with EDTA. When all of the calcium and Magnesium has been complexed by EDTA, the colour of the solution turns blue.

$$Ca^{2+} + Mg^{2+} + \text{Erichromic Black} - T \rightarrow Ca \text{ and } Mg \text{ Erichromic Black} - T \text{ (Wine red)} + EDTA \rightarrow CaEDTA + MgEDTA + \text{Erichromic Blacl} - T \text{ (blue)}$$

(3) Reagents

Buffer solution: Dissolve 67.5gms of NH_4Cl in 570ml of concentration NH_4OH. Dilute to 1000ml in a volumetric flask with distilled water.

Erichromic Black-T indicator: Dissolve 4.5gms of hydroxylamine hydrochloride and 0.50gm of Erichromic Black-T in 100ml of 70% ethanol. This indicator should be prepared fresh every 2 to 3 months.

Standard Calcium Solution, 0.010M: Transfer 1000gm of anhydrous $CaCO_3$ to a 1000ml beaker. Add 1.1HCl slowly to dissolve the $CaCO_3$ and dilute to about 200ml with distilled water. Boil for 5 minutes to expel CO_2, cool and adjust to pH 7, as determined with a pH meter, with 3N NH_4OH. Transfer to a 1000ml volumetric flask and dilute to volume with distilled water.

Standard EDTA titrant: Dissolve 4.00gm of EDTA salt and 100mg of $MgCl_2.6H_2O$ in distilled water to 1000ml. This solution must be standardized against the standard calcium solution. Pipet 10ml of standard calcium solution into a 250ml beaker and add 90ml of distilled water. Add 8 drops of Erichromic Black-T. Titrate the calcium with EDTA.

Compute the normality of the EDTA from equation $NV = N'V'$ (See Total alkalinity).

(4) Procedure

- Measure 100ml of water sample into a 250ml Erlenmeyer flask.
- Add 2.0ml of the buffer solution and mix.
- Add 8 drops of Erichromic Black-T indicator.

- Titrate against EDTA solution. At the end point, the solution will change from wine red to pure blue.
- Calculate the total hardness by following equation.

$$\text{Total Hardness (mg/L as } CaCO_3) = \frac{(\text{ml of EDTA})(M)(100.0)(1000)}{\text{Sample volume in ml}}$$

(5) Comments

Sample for total hardness titration should not be stored for more than 2-3 days.

Note: Total hardness can also be measured partly as Calcium hardness and Magnesium hardness.

(6) Interpretation

Type of water	Hardness range (mg/L)
Soft water	0-60
Medium water	60-120
Hard water	120-180
Very hard water	>180

1.5 Calcium Hardness

(1) Principle

The calcium concentration in water is normally expressed as calcium hardness in terms of equivalent $CaCO_3$. Ethylenediamine tetra acetic acid (EDTA) which complexes Ca^{2+} is used as the titrating agent for calcium hardness. EDTA forms a stable complex with both Ca^{2+} and Mg^{2+},

$$Ca^{2+} + \text{EDTA} \rightarrow \text{CaEDTA}$$

$$Mg^{2+} + \text{EDTA} \rightarrow \text{MgEDTA}$$

Therefore a water sample is made mildly alkaline (pH 12 to 13) to precipitate magnesium as its hydroxide so that the EDTA titration is specific for calcium. Actually, others divalent metals also form stable complexes with EDTA, but Ca^{2+} and Mg^{2+} are the predominant divalent metals in normal natural waters.

The end point of the Calcium hardness titration is detected with murexide. This indicator forms a complex with Ca^{2+} and in the presence of Ca^{2+}, murexide imparts a pink colour to a solution. The complex formed by murexide with Ca^{2+} is not as stable as the complex formed by EDTA with Ca^{2+} . In titration, a small amount of murexide is added to the sample which complexes some of the calcium ions to produce a pink colour. As EDTA is added it reacts with Ca^{2+} in solution to form CaEDTA and, when all of the uncomplexed Ca^{2+} has been titrated, the calcium dissociate from the Ca-murexide complex to form more stable complex with EDTA. The murexide turns orchid purple upon loss of its calcium. The titration is described more simply as follows:

$$\underset{\text{(Pink)}}{Ca^{2+} + Ca - \text{murexide} + EDTA} \rightarrow \underset{\text{(Orchid purple)}}{CaEDTA + \text{Murexide}}$$

(2) Reagents

Sodium hydroxide (NaOH) Solution (1N): Dissolve 40g of NaOH and dilute to 1000ml with distilled water. Store in a tightly rubber stoppered bottle.

Standard EDTA: See hardness.

Murexide indicator: Mix 200mg of murexide indicator (Ammonium purpurate) with 100g of NaCl. Grind with a morter and pestle until grinding cause no further intensification of colour (40-50 mesh). Store the indicator in an opaque bottle.

(3) Procedure

- Measure a 100ml water sample into a 250ml beaker.
- Add 4.0ml of 1N NaOH and stir.
- Add 100 to 200mg of murexide and stir while titrating slowly with standard EDTA. The colour of the solution will gradually change from pink to orchid purple. At the end point, a single drop of EDTA will cause no further increase in the intensity of the colour.
- The volume of the last drop must be subtracted from the burette reading.

(4) Calculation

$$\text{Calcium Hardness } (\text{mg/L } CaCO_3) = \frac{(\text{ml of EDTA})(\text{M})(100.0)(1000)}{\text{Sample volume in ml}}$$

(5) Precaution

The titration must be conducted immediately after the addition of the hydroxide solution and the indicator to the sample.

1.6 Magnesium Hardness

Magnesium hardness may be estimated as the difference between total hardness and calcium hardness as $CaCO_3$ if interfering metals are present in non interfering concentrations in the calcium titration.

$$\text{mg Mg/L} = [\text{Total hardness (as } CaCO_3\text{/L)} - \text{Calcium hardness (as mg } CaCO_3\text{/L)} \times 0.243]$$

1.7 Free Carbon Dioxide (Titrimetric Method with Na_2CO_3)

(1) Principle

Water having a pH value more than 8.34 (here Phenolphthalein end point) does not contain appreciable carbon dioxide dissolved in it. Therefore, the amount of base required to raise the pH of a water sample to the phenolphthalein end point is approximately equivalent to the CO_2 content of the sample.

CO_2 reacts with standard solution (NaOH, a base or Na_2CO_3, a bicarbonate) as follows:

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O \quad (1)$$

$$Na_2CO_3 + H_2O \rightarrow 2NaHCO_3 \quad (2)$$

These two reactions indicate two possible methods for determining CO_2 in water- titration either with standard NaOH or with standard Na_2CO_3 to the phenolphthalein end point. The use of sodium carbonate over NaOH for the titration is that Na_2CO_3 is a primary standard and it is not essential to standardize a solution of Na_2CO_3 before titration.

(2) Reagents

- Phenolphthalein indicator- Dissolve 0.5gm of phenolphthalein in 50ml of 95

% ethyl alcohol and add 50ml CO_2 free distilled water. Add 0.0454N sodium carbonate dropwise until a faint pink colour appears to remove all traces of CO_2 from the indicator.

- Standardize Sodium Carbonate (0.0454N) - Dissolve 2.407gms of anhydrous Na_2CO_3 and dilute to 1000ml with CO_2 free distilled water. This standard solution should be made free each day.

(3) Procedure

- Collect water sample carefully so that it does not come in contact with the atmosphere.
- Analyze for CO_2 immediately after collection or within 2 or 3 hours of sample collection.
- Take 50ml sample and add gently 2-3 drops of phenolphthalein indicator solution.
- If the sample turns pink, the pH is above 8.34 and free CO_2 is essentially absent. If the sample remains colorless, it contains free CO_2.
- Sample containing CO_2 must be titrated rapidly with 0.0454N Na_2CO_3 solution. Stir the sample gently while Na_2CO_3 is added. A faint pink colour which remains for 30 seconds marks the end point.

(4) Calculation

$$\text{Free } CO_2 \text{ in ppm} = \frac{\text{ml of N/44 } Na_2CO_3}{\text{Sample volume in ml}} \times 1000$$

$$\text{Free } CO_2 \text{ in mg/L} = \frac{\text{ml of } Na_2CO_3 \times N \times 22}{\text{Sample volume in ml}} \times 1000$$

1.8 Dissloved Oxygen (Winkler's Method)

(1) Principle

In the basic Winkler procedure (Winkler, 1888), a sample of water is treated with Manganous Sulphate $MnSO_4$, Potassium Iodide KI and NaOH. Under highly alkaline condition, the Mn^{2+} ion is oxidized by molecular oxygen to Manganous dioxide (MnO_2), a brown precipitate,

$$Mn^{2+} + 2OH + {}^{1}/_{2}\, O_2 \rightarrow MnO_2 + H_2O$$

Thus only one half of the oxygen in Manganous dioxide came from molecular oxygen. The H_2SO_4 is added to the sample to dissolve the precipitate and produce acid condition for the oxidation of iodide to iodine by Manganous dioxide. The reaction is:

$$MnO_2 + 2I^- + 4H^+ \rightarrow Mn^{2+} + I_2 + 2H_2O$$

The equation shows that the quantity of I_2 released is proportional to the amount of O_2 originally present i.e. one half of a molecule of O_2 resulted in the release of one molecule of iodine (I_2). The amount of I_2 is estimated by titration with standard Sodium thiosulphate. A starch indicator is used to determine the end point of the titration. As long as iodine is present, the solution is blue. When all the iodine has been titrated the solution becomes colourless.

$$I_2 + \text{Starch} - I_2 + 2Na_2S_2O_3.5H_2O \rightarrow Na_2S_4O_6 + 2NaI + 10H_2O + \text{Starch (Colourless)}$$

The amount of iodine used in reaction is taken to calculate the original Dissolved Oxygen concentration.

(2) Reagent

Manganous Sulphate solution: Dissolve 364gms of $MnSO_4.H_2O$ in distilled water, filter and dilute to 1000ml in a volumetric flask.

Alkali-Iodide-Azide solution: Dissolve 500gms of NaOH and 150gms of KI in distilled water and dilute to 1000ml in a volumetric flasks. Dissolve 10gmsof $NaNO_3$ in 40 ml of distilled water and add to the NaOH-KI solution.

Sodium thiosulphate solution: Dissolve 6.3gms of $Na_2S_2O_3.5H_2O$ in freshly boiled and cooled distilled water and dilute to 1000ml in volumetric flask. Add 5 drops of chloroform as preservative. This reagent must be standardized every few days and stored in the dark.

Concentrated Sulfuric acid: Analytical reagent grade.

Sulfuric acid solution 10%: Add 5ml of concentrated H_2SO_4 to 45ml of distilled water.

Potassium dichromate solution, 0.0250N: Dry 2 or 3gms of $K_2Cr_2O_7$ at 105 ℃ and cool in a volumetric flask to 500ml with freshly boiled and cooled distilled water.

Starch indicator: Add 2gms of soluble starch to 100ml of distilled water in a 250ml beaker. Heat while stirring until transparent and add 0.5ml of formalin as a preservative.

Standardization of Sodium thiosulphate solution: Dissolve 2gms of KI in a 500ml volumetric flask with 100ml of distilled water and add 10ml 10% H_2SO_4 solution. Add 10ml of 0.0250N $K_2Cr_2O_7$ into the flask and place the flask in the dark for 5minutes. Dilute to 250 or 300ml of distilled water. Titrate with sodium thiosulphate solution until a pale straw colour is reached. Add 8 drops of starch indicator and titrate until the blue colour of the starch suddenly disappears. Record the volume of the sodium thiosulphate used and calculate the normality by $NV = N'V'$ (See Total alkalinity).

(3) Procedure

- Collect water sample in a 300ml BOD bottle taking care so that no air buble remains in the bottle.
- Add 2.0ml of Manganous sulphate solution and 2.0ml of alkali-iodide-azide solution below the surface of sampler by dipping the pipette and stoppered with care to prevent air bubble.
- Mix the solution in the bottle by rapidly inverting it around twenty times and then let the sample stand until a precipitate settles to the bottom.
- Add 2.0ml of concentrated H_2SO_4 with a measuring pipette, stopper the bottle carefully, and invert several times to dissolve the precipitate.
- Take 50ml of sample in a volumetric flask.
- Add 4-5 drops of starch solution to it. It turns blue.
- Titrate against 0.025(N) Sodium thiosulfate till blue colour disappears.

(4) Calculation

$$\text{Dissolve } O_2\ (\text{mg/L}) = \frac{\text{ml of sodium thiosulfate} \times N \times 8 \times 1000}{\text{ml of sample titrated}}$$

Note: Factor 8 is multiplied because 1N Sodium thiosulfate is equal to 8 mg of O_2.

1.9 Dissolved Phosphorus (Soluble Orthophosphate)

(1) Principle

In an acid solution, orthophosphate reacts with ammonium molybdate to form an Ammonium phosphate-molybdate complex. The molybdenum in the complex can be reduced to a blue-coloured solution. The intensity of the blue colour formed in this solution increases in proportion to the amount of phosphate present since uncomplexed molybdenum in solution does not react with the reducing agent. A number of reducing agents may be employed, but stannous chloride has been widely used for this purpose in water analysis. Reaction may be summarized as:

$$PO_4^- + 12(NH_4)_2MoO_4 + 24H^+ \rightarrow (NH_4)_3PO_4.12MoO_3 + 21NH_4^+ + 12H_2O$$

The intensity of the blue colour can be measured by spectrophotometer (or colorimeter) and the concentration of orthophosphate is determined by reference to a calibration graph prepared from known concentration of orthophosphate.

(2) Special Apparatus

Suction flask, vacuum source, membrane filter holder for 47mm filters, glass fibre filter (Gelman Type A-E or equivalent, 47mm) and a spectrophotometer (or colorimeter), test tube, glass rod, physical balance, measuring cylinder.

(3) Reagents

Ammonium Molybdate, $(NH_4)6Mo_7O_{24}.4H_2O$ Reagent: Dissolve 25gm of $(NH_4)6Mo_7O_{24}.4H_2O$ in 175ml of distilled water. Cautiously add 280ml of concentrated H_2SO_4 to 400ml of distilled water in a 1000ml volumetric flask and let cool. Add the molybdate solution to the flask and dilute to 1000ml with distilled water.

Stannous Chloride, $SnCl_2$ Reagent: Dissolve 2.5gm of fresh $SnCl_2.2H_2O$ in 100ml of glycerol by heating in a water bath and stirring with a glass rod.

Standard Phosphate-Phosphorus Solution: Dissolve 0.2195gm of KH_2PO_4 in distilled water and dilute to 1000ml in a volumetric flask. This solution is too concentrated to use directly. Make a second solution containing 5.00mg/liter of phosphate-phosphorus by diluting 50ml of the first solution to exactly 500ml with distilled water. (1ml=1μgm of phosphate or 0.001mg of phosphate)

(4) Procedure

- Pipette approximate amount of phosphate solution to cover the range of 0.3-1.5mg/l into a series 100ml nessler tubes or test tubes. Dilute to 100mml using distilled water. Label the tube with the concentrations. These tubes serve as standards. Include one more tube containing 100ml distilled water as the blank.
- Take 100ml of the water sample in a nessler tube.
- To the blank, standards and sample, add 4ml of ammonium molybdate solution and mix well.
- Add 0.5ml stannous chloride to all the tubes and mix well.
- Wait for 10-12 minutes for the development of colour.
- Calibrate the spectrophotometer or colorimeter using blank (instrument reads 100% transmittance or 0% absorbance) and distilled water.
- Measure the intensity of blue coloured complex at 690nm using spectrophotometer.
- Prepare a standard curve by plotting the phosphate concentration of standard solutions on the x axis and the optical density (OD) on the y axis.
- Find the phosphorus content of the sample by matching its absorbance(s) with the standard curve.
- Express the result as mg/l phosphate as phosphorus. If it has to be expressed in terms of phosphates, multiply by a factor of 3.066.

(5) Precautions

- All glassware and containers must be carefully cleaned to prevent contamination with phosphorus. Prior to the initial use, rinse the glassware in 1 to 2N HCl, wash with detergent and tap water, and rinse in distilled water. The detergent must be phosphate free.
- Determine the orthophosphate within 2 to 3 hrs of sample collection. Collection bottles should be carefully chosen so that no additional phosphate is added to the sample. Bottles may be treated with a KI-I_2 solution to inhibit bacterial growth. To treat, fill the bottles with a solution containing 50gm I_2/l and 80gm KI/l and let stand for one week. Pour out the KI-I_2 solution (save this solution for later use) and wash the bottles thoroughly with distilled water. Any discolouration of the sample by I_2 will be destroyed by $SnCl_2$. Bottles may be filled with the KI-I_2 solution and stored until needed again.
- Use distilled water to read zero in the spectrophotometer. The reagent blank

(0.00mg/l Phosphorus) should read 100% transmittance or 0.00% absorbance on the spectrophotometer. If it doesn't, the reagents or distilled water contain phosphorus. If the amount of phosphorus contamination is slight, set the instrument at 100% transmittance with the reagent blank rather than with distilled water.

1.10 Total Phosphate

(1) Principle

The various forms of phosphorous are all hydrolyzed to orthophosphate by treatment with acid, heat and pressure. Orthophosphate is then measured by stannous chloride method.

(2) Special Apparatus

All materials required for the determination of orthophosphates; in addition, kjeldahl flask, volumetric flask, burette, Bunsen burner, tripod stand and wire gauge.

(3) Reagent

1N Sodium hydroxide: Dissolve 40gm of NaOH pellets in about 200ml of distilled water. Make up the volume to 1000ml in a volumetric flask.

Phenolphthalein: Dissolve 0.50gm of phenolphthalein in 50ml of 95% ethyl alcohol and 50ml of distilled water. Add 0.02N NaOH dropwise until a faint pink colour appears.

(4) Procedure

- Take 100ml of the sample in a kjeldahl flask.
- Add carefully 1ml of concentrated H_2SO_4 and 5ml concentrated HNO_3.
- Heat the sample until the solution becomes colourless.
- Cool and add 20ml of distilled water and 2 drops of phenolphthalein indicator.
- Titrate against sodium hydroxide until the appearance of a pale pink colour.
- Transfer the solution to a 100ml volumetric flask and dilute it upto the mark.
- Determine the total phosphates by the same procedure as described for orthophosphates.

1.11 Dissolved Nitrate (Brucine Sulphonic Acid Method)

(1) Principle

It is based on the reaction of nitrates of brucine sulphate in strong acidic condition. The intensity of colour produced by the reaction is proportional to the concentration of nitrate originally present in the sample, permitting nitrate analysis by spectrophotometer. The concentration of nitrate-nitrogen is estimated by reference to a calibration graph. The calibration graph is prepared plotting transmittance or absorbance values on the y axis versus their respective concentrations of nitrate-nitrogen on the x axis.

(2) Special Apparatus

A spectrophotometer, Whatman No 42 filter paper, Volumetric flask, Pipette, Beaker.

(3)Reagent

Coupling reagent: Dissolve 500mg of N-(I-napthyl)-ethylenediamine dihydrochloride in 500ml of distilled water. Store in a dark bottle and keep out of the light. This reagent gradually becomes dark brown and must be prepared fresh every 2 to 4 weeks.

Diazotizing reagent: Add 5g of Sulphanilamide and 50ml of concentrated hydrochlorine acid to 300ml of distilled water in a 500ml volumetric flask. Stir to dissolve and then dilute to volume.

Standard Nitrite-Nitrogen Solution (1.00mg/L): Dissolve 0.4925g of $NaNO_2$ in 1000ml of distilled water. This solution contains 100mg/L of NO_2-N. Pipette 1000ml of the 100mg/L NO_2-N solution into a 1000ml volumetric flask and dilute to volume with distilled water to give a 100mg/L NO_2^--N solution. These solutions deteriorate rapidly.

(4) Procedure

- Filter the water sample through Whatman No 42 or equivalent filter paper.
- Measure 50ml of it in a 100ml beaker.
- Add 1.0ml of coupling reagent and stir.
- Let the solution stand for 10 minutes to form the azo compound.
- Transfer to a cuvette and measure the pink colour by a spectrophotometer at 543nm.

- Use a reagent blank to set the spectrophotometer at 0.0 absorbance (100% transmittance).
- Prepare a series of NO^{2-}-N concentration from standard solution. For this, take 2.0, 4.0, 6.0, 8.0, 10.0, 15.0 and 20.0ml of 1.0mg/L NO^{2-}-N solution in 25ml test tube and add reagent as in the sample. This will give a NO^{2-}-N concentration of 0.02, 0.04, 0.06, 0.08, 0.10, 0.15 and 0.20mg/L respectively.
- Evaluate the pink colour at 543nm.
- Use 0.0mg/L solution to set the spectrophotometer to 0.0 absorbance or 100% transmittance.

(5) Calculation

$$\text{mg/L } NO_2^- - \text{N} = \frac{\text{ml } NO_2^- - \text{N} \times 1000}{\text{sample taken for estimation}}$$

$$\text{mg/L } NO_2^- = 3.28 \times \text{mg/L } NO_2^- - \text{N}$$

1.12 Sulphate Estimation

(1) Principle

Sulphate ion is precipitated in an Acetic acid medium with Barium chloride ($BaCl_2$) so as to form Barium sulphate, $BaSO_4$ crystals. Light absorbance of the $BaSO_4$ suspension in measured using spectrophotometer and the SO_4^{2-} concentration is determined by comparing with a standard curve. The minimum detectable concentration is 1mgSO_4^{2-}/L.

(2) Special Apparatus

Magnetic stirrer, Spectrophotometer, Stopwatch or electric timer, Measuring spoon (0.2 to 0.3ml).

(3) Reagents

Buffer Solution A: Dissolve 30g of Magnesium chloride $MgCl_2.6H_2O$, 5g Sodium acetate, $CH_3COONa.3H_2O$, 1.0g potassium nitrate, KNO_3 and 20ml acetic acid, CH_3COOH (99%), in 500ml distilled water and make up to 1000ml.

Buffer Solution B (Required when the sample contain less than 10mg SO_4^{--}

/L): Dissolve 30g of $MgCl_2.6H_2O$, 5g $CH_3COONa.3H_2O$, 1.0g KNO_3, 0.111g Sodium sulphate, Na_2SO_4 and 20ml Acetic acid (99%) in 500ml distilled water and make upto 1000ml.

Barium Chloride, $BaCl_2$.

Standard Sulphate Solution: Prepare a standard sulphate solution as described in (1) or (2) below:

1. Dilute 10.4 ml standard 0.0200N H_2SO_4 titrant to 100ml with distilled water.
2. Dissolve 0.1479g anhydrous Na_2SO_4 in distilled water and dilute to 1000ml.

$$\text{It gives an estimation of } 1.00\text{ml} = 100\mu\text{g } SO_4^{2-}$$

(4) Procedure

- Measure 100ml sample or a suitable portion made up to 100ml into a 250ml Erlenmeyer flask.
- Add 20ml of buffer solution A and mix in stirring apparatus.
- While stirring add a spoonful of $BaCl_2$ crystals and begin timing immediately. Stir for around 60s at constant speed.
- After stirring period has ended, measure turbidity.
- Estimate SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO_4^{2-} standards through the entire procedure.

(5) Precautions

- Space standards at 5mg/L increments in the 0 to 40mg/L SO_4^{2-} range. Above 40mg/L accuracy decreases and $BaSO_4$ suspensions lose stability.
- Check reliability of calibration curve by running a standard with every three or four samples.

(6) Calculation

$$\text{mg } SO_4^{2-}/\text{L} = \frac{\text{mg } SO_4^{2-} \times 1000}{\text{ml sample}}$$