# DRAFT MONITORING AND ANALYIS PROTOCOL FOR COASTAL WATERS UNDER ICZM PROJECT

## BACKGROUND

Gujarat is having a coastal belt of about 1600 Kms which is throbbing with variety of developmental and industrial activities and hence this belt and the nearby area is one of the major sources of economy of the state. Apart from the primary fishing activities and the ancillary industries, it also provides employment to the people in the area. Coastal area play vital role for the growth of fishermen community and also for the growth of developmental projects especially which are dependent on marine resources. Because of all these activities, there always remain a pollution potential of the coastal waters. To know the pollution level and its effect on marine life, monitoring of coastal area is vital. On the basis of analysis and subsequent interpretation of analytical data, pollution load in coastal area can be estimated. Physico-chemical analysis and biological analysis is required to assess the quality of coastal water. These parameters are in turn related to the chemical composition of sea water and its monitoring and analysis help us to control pollution level at different sources. Deciding suitable and appropriate monitoring locations is therefore one of the most important aspects for the real evaluation and assessment of environmental parameters.

## MONITORING TEAM

Monitoring of Gulf of Kutch waters is under taken by the Scientists form Regional Offices viz. Jamnagar, Bhuj and Rajkot in consultation with concerned Regional officers and with the Nodal Officer ICZMP at Head Office, Gandhinagar.

## LOCATIONS

Sampling locations are decided in consultation with concerned Regional Officers of the Board and the Nodal Officer, ICZMP at Head Office. After completion of selection of all locations, the details were submitted to S.P.M.U and all PEA's for their suggestions & recommendation before implementation of sampling programme under ICZM Project. Following criteria has been considered for selection of sampling stations:

- Industrial activities and developmental activities in surrounding area.
- Direct or indirect discharge of waste water into the sea.
- Urbanization / human activities in surrounding area.
- Approachability to the stations by road in all types of weather.
- Availability of boat for sample collection.
- Tourism/ Port activities.

## MONITORING AREA

During selection of sampling points, we have considered both 'Source' and 'Sink' and hence Source monitoring as well as Sea water monitoring is incorporated in the monitoring protocol. The 'Source' sampling locations includes Domestic outfalls / Industrial discharges in the area where as 'Sink' sampling locations includes sampling of receiving sea water into which the Domestic out falls / Industrial discharges have mixed. All, three regions viz. Rajkot, Jamnagar and Kutchh are included for monitoring.

### A. Source Monitoring

| Sr. No. | Industrial Cluster/Area/Other Activity | Longitude & Latitude |
|---|---|---|
| **Regional office: Jamnagar** | | |
| 1 | Jamnagar Municipal Corporation Domestic Outfall waste water from pumping station of Gandhinagar, Jamnagar | 22°29'477"N 70°03'318"E |
| 2 | Cooling water return through open channel water sample of GEB TPS – SIKKA | 22°25'121"N 69°49'201"E |
| 3 | Essar Oil Limited, (Sea Water Basin Outlet) | 22°20'606"N 69°45'056"E |
| 4 | From Final Outlet of Tata Chemicals, Mithapur, (after dilution after settling) | 22°24'640"N 69°02'066"E |
| 5 | Reliance Industries Ltd., (Sea water Basin Outlet) | 22°26'631"N 69°50'621"E |
| **Regional office: Bhuj** | | |
| 1 | Confluence point of Tata Power & Adani Power, Mundra, Tal. : Mundra | 22°48'08.82"N 69°32'12.38"E |
| 2 | Domestic waste water sample of outlet of KSEZ/Gandhidham city nr. kidana | 23°01'03.36"N 70°07'27.28"E |

### B. Sea Water Monitoring

| Sr. No. | Industrial Cluster/Area/Other Activity | Longitude & Latitude |
|---|---|---|
| **Regional office: Jamnagar** | | |
| 1 | Sea Water sample at Rozy Port, Near Light House | 22°34'171"N 70°03'421"E |
| 2 | Sea Water sample at Sachana Ship Breaking Yard | 22°34'363"N 70°10'918"E |
| 3 | Sea water sample @ 2 km. away from RIL Jetty. | 22°31'277"N 69°48'590"E |
| 4 | Sea water sample @ 2 km. away from Essar Jetty. | 22°26'208"N |

| | | |
|---|---|---|
| | | 69°40’515”E |
| 5 | Sea water sample of Tata Chemicals Ltd., Arambhda | 22°26’167”N<br>69°02’572”E |
| **Regional office: Bhuj** | | |
| 1 | Sea water sample @ 300 meter away from old mundra port, Tal. : Mundra | 22°43’43.40”N<br>69°42’10.54”E |
| 2 | Sea water sample @ 300 meter away from new mundra port, Tal. : Mundra | 22°44’14.37”N<br>69°43’11.90”E |
| 3 | Sea Water sample at Mandvi Port, Mandavi, Tal. : Mandvi | 22°49’21.70”N<br>69°21’04.16”E |
| 4 | Sea water sample @ 300 meter away from Kandala Port, Tal. : Gandhidham | 23°00’27.32”N<br>70°13’21.37”E |
| **Regional office: Rajkot** | | |
| 1 | Sea water sample at Navlakhi port | 22°57’33.1”N<br>70°26’38.1”E |
| 2 | Sea water sample of Surajbari creek (Road side) | 23°11’43.2”N<br>70°42’59.5”E |

The Prominent Sampling Locations thus covers Industrial Outfalls, Treated effluent drainage, Port activities and other activities in the adjoining area (land mass) of the Gulf of Kutchh.

## PARAMETERS MONITORED

Presently, the physico chemical parameters monitored are pH, DO, COD, BOD, Electrical Conductivity in µmhos/cm, ($NO_2$+$NO_3$)-Nitrogen, Suspended Solids, Faecal Coliform (MPN/100 ml), Total phosphorous, TKN, Total Ammonia ($NH_4$+$NH_3$)-Nitrogen, Salinity, Phenols, Oil and Grease and Heavy metals are monitored.

Monitoring and analysis of Parameters like Dissolved / dispersed hydrocarbons in sea water, Chlorinated hydrocarbons, Pesticide residues, Bottom sediments (for Heavy Metals), Particulate organic carbon (POC) and particulate nitrogen (PN), Dissolved metals and ions, Petroleum hydrocarbons and Total organic carbon would be carried out after the Sophisticated Analytical Instruments are Procured under the Project and Installed at various Laboratories of the Board.

## FREQUENCY OF SAMPLING

Monitoring of coastal waters from above locations has been started from October 2010 with frequency of sampling once in a month for each monitoring location. It has been decided during meeting with S.P.M.U that first year data will be a base line data.

## METHOLOGY USED FOR SAMPLING

Niskin water samplers are used in the field to collect Surface and Bottom water samples at each station. Similarly Sediment Grab sampler would be utilized for collection of sediment samples from the bottom. GPSs are utilized to precisely fix the sampling stations with respective Latitudes and Longitudes. This helps collecting the water / sediment samples from the same locations each time. Moreover,

For depth < 5 meters, only surface water sample is to be collected; for depth between 5 to 10 meters both surface and bottom water samples are to be collected whereas for depth > 10 meters surface, middle and bottom water samples are to be collected.

Parameters like B, F and CN are to be analysed only if there is an expected source, otherwise they can be dropped. COD is to be done only for 'Source' stations and not for 'Sink' Stations. Silica is to be done for all stations i.e. new parameter Silica/silicates is to be incorporated for analysis.

## METHOLOGY USED FOR ANALYSIS

Methods prescribed in Standards Methods for the Examination of Water and Waste Water prepared and published by APHA, AWWA are used by the Regional Offices of the Board for analysis of sea water samples. However, Coastal Water Quality Measurements Protocol for COMAPS programme prepared by Integrated Coastal and Marine Area Management (ICMAM) Directorate, Chennai is also referred and incorporated appropriately in this Draft Protocol. The list of references for various analytical methods appears at the end of this draft protocol.

## COLOR

**METHOD:-** Visual Comparison Method

**COLLECTION OF SAMPLE:** Collect the sample in the plastic carbouy.

**PRESERVATON :** Ice.

**APPARTUS:-** Color Comparator, Color Disks.

**REAGENTS:-** Not Applicable

**PROCEDURE:-** Observe sample color by filling a matched nessler tube to the 50-ml mark with sample and comparing it with standards. Look vertically downward through tubes toward a white or spectral surface placed as such an angle that light is reflected upward through the columns of liquid. If turbidity is present and has not been removed report as "Apparent Color". If the color exceeds 70 units, dilute the sample with D.W. in known proportion until the color is within the range of the standards.

**CALCULATION:-** Color unit Pt. Co. Scale = Reading x Dilution

**INTERFERENCE:-** Turbidity

**PRECISION & BIAS:-** The color value of water is extremely pH dependent and invariably increases as the pH of the water is raised. When reporting a color value, specify the pH at which color is determined.

## TEMPERATURE $^{o}$C

**METHOD :-** A calibrated thermometer is allowed to stand in seawater sample and the reading is recorded.

**APPARTUS :-** Certified thermometer: 0-50°C with 0.1° accuracy
Thermometer: 0-50°C having mercury thread, calibrated with the certified thermometer.

**PROCEDURE :-** Dip the thermometer in the seawater sub-sample drawn for temperature measurements. This sub-sample to be drawn immediately after retrieving the samples onboard. Record the temperature after 2 min.

**Results:** Report the reading in $^{o}$C.

# TURBIDITY

**METHOD:-** Nephelometric

This method is based on a comparison of the intensity of light scattered by a sample and a standard reference under same condition. Higher the intensity of scattered light higher the turbidity.

**COLLECTION OF SAMPLE:-** Collect the sample in the plastic carbouy

**PRESERVATION:** Ice

**APPARATUS:-** Nephelo Turbidity Meter

**REAGENTS:-**
**Soultion 1:-** Dissolve 1.0 gm Hydrazine Sulfate and dilute to 100 ml with distilled water.

**Solution 2:-** Dissolve 10 gm hexamethylene tetra mine and dilute to 100 ml.

**Solution 3:-** Mix 5.0 ml of solution 1 with 5.0 ml of solution 2. Allow it to stand for 24 hours and dilute to 1000 ml. This will have turbidity of 400 units.

**Standards turbidity suspension:** Dilute 10ml of solution 3 as prepared above to 100 ml to have turbidity of 40 units.

**PROCEDURE:-** Switch on the instrument and select calibration curve for desired range. Place the filter frame in position. Fill the sample in clean and dry turbid meter tube up to the mark and then lower the plunger into the sample tube carefully, Place it in the circular groove of the mirror tube. Close the door of the apparatus. Switch on the ON/OFF switch. Immediately balance the light intensity of the central spot with the surrounding field with the dial knob and read the scale on the dial.

**CALCULATION:-** Determine the turbidity directly from the selected graph and it dilution is made multiple by dilution factor.

**INTERFERENCE:-** Dirty glassware, air bubbles, debris and the coarse sediments.

**PRECISION & BIAS:-** When comparing water treatment efficiencies, do not estimate turbidity more closely than specified. Uncertainties and discrepancies in turbidity measurement make it unlikely that result can be duplicated to greater precision than specified.

# pH

**METHOD** :- Electrometric

The pH is determined by measurement of the electromotive force of a cell comprising an indicator electrode, an electrode responsive to hydrogen ions (glass electrode) immersed in the test solution of a reference electrode. Contact between the test solution of the reference solution is achieved by means of a liquid junction, in the reference electrode. A difference of 1 pH unit produces a potential change of 58.16 mv at 25°C. The electromotive force is measured with a pH to read directly as pH value.

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy.

**PERSERVATION : -** Ice

**APPARATUS:-** pH Meter, Glass Electrode, Reference electrode (Combined electrode).

**REAGENTS :-** Std. Buffer solution. of pH-7, pH-4 & pH-9. This can be prepared from buffer tablets, which are commercially available or readymade standard solutions of pH 4, 7 and 9.

**PROCEDURE :-** Rinse the electrode with Distilled Water and dry by gentle wiping with a soft tissue paper. Standardize the instrument with the electrodes immersed in a Buffer solution with a pH close to that of the water sample to be tested.

Take different Samples in beaker and put the electrode in baker and note down the stable reading at relative temperature.

**CALCULATION :-** Instrument gives direct reading.

**INTERFERENCE :-** The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductions, or high salinity, except for a sodium error at pH >10. Reduce this error by using special “low sodium error” electrodes. pH measurements are affected by temperature. The Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

**RESULT :-** Report temperature at which pH is measured.

**PRECISION & BIAS :-** By careful use of a Laboratory pH meter with good electrodes, a precision of ± 0.02 pH unit and an accuracy of ± 0.05 pH unit can be achieved. However, ± 0.1 pH unit represents the limit of accuracy under normal conditions, especially for measurement of water samples and poorly buffered solutions. For this reason report pH values to the nearest 0.1 pH unit.

# CONDUCTIVITY

**METHOD:-** Instrumental Method

**PRINCIPLE:-** It is defined as the ability of an aqueous solution to carry an electric current. This Ability depends on the presents of ions, on their total concentration, mobility, and Valance and on the temperature of instrument.

**COLLECTION OF SAMPLE:-** Collect the sample in the plastic carbouy.

**PRESERVATION:-** Ice

**APPARATUS:-** Conductivity Meter

**REAGENTS:-**

1) 0.1 M KCL → 7.456 GM 1 Liter distilled water
2) 0.01 M KCL → 0.1 Mm KCL 10ml → make up to 100 ml with distilled Water
3) 0.001 M KCL → 0.01 M KCL 10 ml → make up to 100 ml with distilled Water.

**PROCEDURE:-**

**DETERMINATION OF CELL CONSTANT** Rinse the conductivity cell with at least three portions of 0.01 M KCL solution. Adjust the temp of a fourth portion to 25 $\pm$ 0.1 ° C. Measure resistance of this portion and note the temperature. Compute cell consent, C.

**CALCULATION:-**

$$\text{Cell Consent C} = \frac{\text{Specific conductance of N/ 100 KCL solutions in µmhos/ cm}}{\text{Measured conductance of N /100 KCL solutions in µmhos /cm}}$$

It is easy to calculate the cell constant from the measurement of the conductance (or Resistance) of a solution of known "specific conductance"

$$\text{Specific conductance (Mhos/cm of the Sample at 25 °C)} = \frac{\text{Cell Constant}}{\text{measured resistance in ohms}}$$

**INTERFERENCE:-** The Conductivity result mainly depends on the cell constant and hence cell error in constant determination will cause error in conductivity.

**PRECISION & BAIS:-** The precision of commercial conductivity meter is commonly between 0.1 to 1.0 % reproducibility of 1 to 2 % is expected after an instrument has been calibrated.

# TOTAL SUSPANDED SOLIDS

**METHOD:-** Filtration Method

**COLLECTION OF SAMPLE :-** Collect the sample in plastic Carbouy.

**PRESERVATION :-** Ice

**APPARATUS :-**

a) Conical flasks
b) Funnel
c) Measuring Cylinder
d) Glass fiber filter paper (Whatman GF/c 47 mm diameter circles)
e) Vacuum Assembly
f) Oven

**REAGENTS :-** Not Applicable

**PROCEDURE:-** Assemble filtering apparatus & filter and beign suction wet filter with a small volume of distilled water to seal it. Filter a measured volume well mixed sample through the glass fiber filter paper. Wash with three successive 10 ml volume of distilled water, allowing complete drainage between washing and continue suction for about three minutes after filtration is complete. Carefully remove filter from filtration apparatus with the help of clean forceps and transfer to Petri dish. Dry for at least 1 hour at 103 °C to 105 °C in an oven, cool in desiccators before weighing.

Repeat the cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until the weight loss is less than 4 % of the previous weight, or 0.5 mg whichever is less.

**CALCULATION ;-**

$$\text{TSS mg/l} = \frac{(B - A) \times 10^6}{\text{Sample taken (ml)}}$$

Where,

A = Weight of empty filter paper

B = Weight of filter + dried residue

**PRECISION AND BAIS :-** Single laboratory duplicate analysis of 41 samples of water and waste water were made with a standard deviation of differences of 6.0 mg/l.

# TOTAL DISSOLVED SOLIDS

**METHOD:-** Filtration Method

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION:-** Ice

**APPARATUS:-**

1. Conical flask
2. Funnel
3. Beaker
4. Measuring Cylinder
5. Oven
6. Balance

**REAGENTS:-** Not Applicable

**PROCEDURE:-** Filter measured volume of well mixed 50 ml sample through glass fiber filter, wash with three successive 10 ml volumes of distilled water, allowing complete drainage between washings and continue suction for about 3 minutes after filteration is complete. Transfer filtrate to a weight evaporating dish or a beaker and evaporate to dryness on a steam bath. If filtrate volume exceeds dish capacity add successive portions to the same dish after evaporation. Dry for at least 24 hour in an oven at 180 °C $\pm$ 2 °C, cool in a desiccator before weighing. Repeat drying cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until weight lost is less than 4 % of pervious weight or 0.5 mg. whichever is less.

**CALCULATION:-**

$$\text{TDS mg/l} = \frac{(A - B) \times 1000}{\text{Sample taken (ml)}}$$

Where,
A= Weight of dried residue + beaker or evaporating dish (crusible)
B= Weight of empty beaker or evaporating dish (crusible)

**INTERFERENCE:-** N.A.

**PRECISION AND BAIS:-** Single Laboratory analysis of 77 samples of a known of 293 mg/l were made with a standard deviation of differences of 21.20 mg/l.

# CHLORIDE

**METHOD :-** Argentometric method (Titrimetric method)

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy.

**PRESERVATION :-** Ice

**APPARATUS :-** Conical Flasks, Burette, Pipette, Funnels

**REAGENTS :-**

1. Standard $AgNO_3$ Solution (Titrant) (0.0282 N) :- 4.79 gm $AgNO_3$ in 1 liter distilled water.
2. Standard NaCl (dried at 140 °C) 1.648 gm in 1000 ml distilled water.
3. Potassium Chromate ($K_2CrO_4$) Indicator Solution:- 50 gm $K_2CrO_4$ + little volume of distilled water. Add $AgNO_3$ until definite red precipitates appears, let it stand for 12 hours, then filter & dilute with 1000 ml distilled water.

**PROCEDURE :-** Take pretreated sample (Adjust to neutral pH) & add 1 ml Potassium chromate indicator & titrate against 0.0282 N Silver nitrate.
Also run the Charcoal / reagent blank. Color change – yellow to reddish orange.
In case of coloured sample, give charcoal treatment /3 ml of $Al(OH)_3$ suspension to remove colour from sample and then filter it.

**CALCULATION :-**

$$\text{Chloride } (Cl^-) \text{ mg/l} = \frac{\text{B.R.} \times \text{Normality of silver nitrate X } 35450}{\text{Sample Taken (ml)}} \times \text{Dilution}$$

**INTERFERENCE :-** Sulfide, Thiosulfate, Sulfite ions, and Orthophosphate in excess of 25 mg/l, Iron in excess of 10 mg/l interferes.

**PRECISION AND BIAS :-** A synthetic sample containing 241 mg CI/L, 108 mg Ca/l, 82 mg Mg/L, 3.1 mg – K/L, 19.9 mg Na/L, 1.1 mg $NO_3$- N/L, 0.25mg $NO_2$-N/L, 259 mg $SO_4$/L and 42.5 mg. Total alkalinity/L (Contributed by $NaHCO_3$) in distilled water was analysed in 41 laboratories by the Argentometric Method with a relative standard deviation of 4.2 % and a relative error of 1.7%.

## OXYGEN (DISSOLVED)

**METHOD :-** Winkler 's Method – Azide modification

Dissolved Oxygen is the quantity of oxygen dissolved in water/ wastewater

**COLLECTION OF SAMPLE :-** a) Collect the sample in 300 ml BOD bottle. b) Avoid contact with air and agitation.

**PRESERVATION :-**

a Add 2 ml $MnSO_4$ (Manganese Sulphate) solution
b Add 2 ml Alkali iodide azide solution
c Shake well and allow settling (brown ppt.)
d Store at low temperature

**APPARATUS :-** BOD Bottles, Burette, Pipette, 100ml measuring cylinder and 250 ml Flask.

**REAGENTS :-**

a. Manganese Sulphate solution: Dissolve 480 gm $MnSO_4$ $4H_2O$ or 400 gm $MnSO_4$ or 364 gm $MnSO_4$ in 1 lit distilled water.
b. Alkali – Iodide Azide regent: Dissolve 500 gm NaOH (or 700 gm KOH) + 135 gm NaI (or 150 gm KI) in 1 liter distilled water + 10 ml $NaN_3$ (Sodium Azide) in 40 ml of Distilled Water.
c. Starch solution:
d. Conc. $H_2SO_4$
e. 0.1 N $Na_2S_2O_3$ (Std. $Na_2S_2O_3$ Stock solution): 24.82 gm $Na_2S_2O_3$ $5H_2O$
f. 0.0125 N $Na_2S_2O_3$ Working Standard Solution: 125 ml stock solution & make upto 1 liter with distilled water.

**PROCEDURE :-** Add 2 ml conc. $H_2SO_4$ mix to dissolve brown precipitate and take 100 ml out of 300 ml in 250 ml flask & titrate with 0.0125 N $Na2S_2O_3$. When pale straw color resists add 1-2 ml starch solution and continue the titration to the first disappearance of the blue color & read the final burette reading.

**CALCULATION :-** 1 ML 0.0125 N $Na_2S_2O_3$ = 0.1 mg D.O.

$$\text{D.O.} = \frac{0.1 \times \text{ML reading} \times 1000}{100 \text{ ml (sample)}}$$

**INTERFERENCE :-** Reducing or oxidizing materials should be absent.

**PRECISION AND BIAS :-** D.O. can be determined with a precision expressed as a standard deviation, of about 20 $\mu$g/L in the distilled water and about 60 $\mu$g/L in waste water and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 $\mu$g/L. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification of the method.

# BIOCHEMICAL OXYGEN DEMAND

**METHOD** :- Winker's Azide modification Method.

BOD is defined as the Oxygen required by the living organisms in the utilization and stabilization of Oxygen matter present in the wastewater.

**COLLECTION OF SAMPLE :-** Collect the sample in 300-ml BOD bottles. Avoid contact with air and agitiation.

**PRESERVATION :-** Ice.

**REAGENTS:-**

1. Phosphate Buffer Solution:-
   8.5 gm $KH_2PO_4$ (Potassium Dihydrogen Orthophosphate) + 21.75 gm $K_2HPO_4$ (Di-potassium Hydrogen Orthophosphate) + 33.4 gm $Na_2HPO_4.7H_2O$ (Disodium hydrogen phosphate) + 1.7 gm NH4Cl (Ammonium chloride) → Make up volume to 1 liter with distilled water.

2. Magnesium Sulphate solution ($MgSO_47H_2O$):-
   Dissolve 22.5 gm $MgSo_4$ $7H_2O$ in 1 liter distilled water.

3. Calcium Chloride Solution (CaCl2):-
   Dissolve 27.5 gm CaCl2 in 1 liter distilled water

4. Feric Chloride Solution (FeCL3 6H2O):-
   Dissolve 0.25 gm FeCl3 6H2O in 1 liter distilled water.

5. Acid – alkali solution 1N:-
   (A) Dilute 28 ml Conc. H2SO4 in 1 liter distilled water.
   (B) Dissolve 40 gm NaOH in 1 liter distilled water.

6. Glucose – Glutamic Standard Solution:-
   150 gm Glucose + 150 mg Glutamic acid in 1 liter distilled water.

7. Manganese Sulphate Solution ($MnSO_4$) :-
   480 gm MnSO4 4H2O or 400 gm MnSO4 or 364 gm MnSO4 H2O in 1 liter distilled Water.

8. Alkali – Iodide – Azide Solution :-
   500 gm NaOH or (700 gm KOH) + 135 gm NaI or (150 gm KI) in 1 liter distilled water + 10 ml $NaN_3$ (sodium azide) in 40 ml distilled water.

9. Conc. $H_2SO_4$:-

10. Starch Solution:-
2.0 gm starch + 0.2 gm salicylic acid as a preservative in 100 ml hot distilled water.

11. 0.0125N $K_2Cr_2O_7$ (Potassium Di- Chromate) :-
0.613 gm $K_2Cr_2O_7$ (Potassium Di- Chromate) in 1 liter distilled water.

12. 0.1 N $Na_2S_2O_3$ (Sodium Thiosulphate) :-
24.82 gm $Na_2S_2O_3$ $5H_2O$ in 1 liter distilled water.

13. Working Sodium Thiosulphate Solution:-
0.0125 N $Na_2S_2O_3$ 125 ml stock solution & make upto 1 liter distilled water.

**PROCEDURE :-** Prepare dilution water by adding 1 ml each reagent 1,2,3,4, per liter in air Saturated distilled water. Add 2 ml seed per liter, make two sets of several Dilution (800/ COD) of the prepared samples, on one set determine an immediate oxygen demand. Incubate at 27°C the blank dilution water and second set after 3 days measure the oxygen left as per dissolve oxygen estimation method.

**CALCULATION :-**

$$\text{BOD mg/l} = \frac{\text{Actual Difference in D.O.} \times 100}{\text{Sample Taken (ml)}}$$

Actual Difference = (0 Day D.O. – 3 Day D.O) – Blank difference

**INTERFERENCE :-** pH of the sample should be adjusted by adding alkali or acid in the range of 6.5 to 8.5. All samples may be sterile and will need seeding. For the purpose of oxidizing the organic matter where such microorganisms are already present as in surface water, domestic sewage or unchlorinated effluents etc. seeding is not required.

**PRECISION AND BIAS :-** The working range is equal to the difference between the maximum initial D.O. (7 to 9) and minimum D.O. residual of 1 mg/l multiplied by the dilution factor. A lower detection limit of 2 mg/l is established by the requirement for a minimum D.O. depletion of 2 mg/l.

For the 300 mg/l mixed primary standard, the average 3 Day BOD would be 198 mg/l with a standard deviation of 30.5 mg/l.

## OIL AND GREASE

**METHOD :-**Partition – Gravimetric Method

**COLLECTION OF SAMPLE :-** Collect the sample in glass bottle.

**PRESERVATION :-** Conc. HCL (to acidity sample)

**APPARATUS :-** Hot plate , Separating funnel

**REAGENTS :-**

1. Hexane :-
   50 ml per sample
2. Sodium Sulphate (anhydrous $Na_2SO_4$) :-
   To absorb moisture
3. Sodium Chloride (NaCl):-
   For emulsion.

**PROCEDURE :-** Take appropriate sample for analysis in the separating funnel (pH of sample should be less than 2). Add 50 ml / 100 ml hexane to the sample. Mix well the sample in separating funnel. Hexane soluble Oil & Grease will be separated from wastewater. Filter the Hexane by 42 no. of filter paper (through $Na_2So_4$) in a dry pre weighed (A) beaker. Put on the hot plate at 65 to 70° C to evaporate the Hexane. Afterwards cool it in desiccators and weight the beaker having oil & grease residues (B).

**CALCULATION :-**

$$\text{Oil \& Grease mg/l} = \frac{\text{Weight different (B - A)} \times 10^6}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** Any filterable organic soluble substances will be extracted along with Oil & Grease. Such as elemental, Sulfur and certain organic dyes.

**PRECISION AND BIAS :-** This method was tested by a single Laboratory on a raw sewage sample using both the extraction solvents. By this method the oil & grease concentration was 20.8 mg/L with trichlorotrifluoroethane and 22.4 mg/L. With the 80:20 hexane / methyl – tert-butyl ether mixture. When sample were dosed with 30 mg Fisher Heavy Mineral Oil, recovery of added oil was 78.9 % with a standard deviation of 0.8 mg/L. for trichlorotrifluoroethane and 84.2% with a standard deviation of 1.2 mg/L for hexane / methyl-tert-butyl ether.

## PHENOLIC COMPOUND

**METHOD :-** Colorimetric method (4- Amino Antipyrine method)

**COLLECTION OF SAMPLE :-** Collect the sample in the glass bottles.

**PRESERVATION :-** $CuSO_4$ + Orthophosphoric acid

**APPARATUS :-** Spectrophotometer, Phenol distillation unit. Nessler tubes

**REAGENTS :-**

1) 0.5N Ammonium hydroxide ($NH_4OH$):-Dilute 35 ml fresh Conc. $NH_4OH$ & make up to to 1000 ml distilled water.

2) Phosphate Buffer Solution:- Dissolve 104.5 gm $K_2HPO_4$ + 72.3 gm $KH_2PO_4$ in 1 liter with distilled water.

3) 4- Aminoantipyrine Solution :-Dissolve 2.0 gm 4 – Aminoantipyrine in 100 ml distilled water.

4) Potassium Fericyanide Solution:- Dissolve 8.0 K3Fe (CN)6 in 100 ml distilled water.

**PROCEDURE :-** Distillate the preserved samples. Take appropriate ml of distilled sample make it 100ml. Add 2.5 ml $NH_4OH$ soln. and Add 1 ml Phosphate buffer solution. Check the PH@ 7.9 to 9.0 then Add 1 ml 4 – Amino Antipyrine solution & Add 1 ml $K_3Fe(CN)_6$, Mix well and let dark red color developed (residue) for 15 minutes then measure O.D. at 500 nm.

It is necessary to make standard curve before analyzing the sample. Also run the reagent blank.

**CALCULATION :-**

$$\text{Phenol mg/l} = \frac{\text{O.D} \times \text{Factor}}{\text{Sample Taken (ml)}} \times \text{Dilution}$$

**INTERFERENCE :-** Interference such as phenol decomposing bacteria, Oxidizing & reducing Substances and alkaline pH values are dealt with by acidification. Some highly contaminated wastewater may require specialized technique for eliminating interference and for quantitative recovery of Phenolic Compounds.

**PRECISION AND BIAS :-** Because the ‘ PHENOL’ value is based on $C_6H_5OH$, this method yields only an approximation and represents the minimum amount of phenols present. This is true because the phenolic reactivity to 4 – aminoantipyrine varies with the types of phenols present.
In a study of 40-refinery wastewater analysed in duplicate at concentration from 0.02 to 6.4 mg/L. The average relative standard deviation was ± 12%. Data are not available for precision at lower concentration.

# HEXAVALENT CHROMIUM

**METHOD :-** Colorimetric Method (Diphenylcarbazide)

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION :-** Conc. $HNO_3$

**APPARATUS :-**Spectrophotometer

**REAGENTS :-**

1. Stock Chromium Solution :- Dissolve 141.4 mg $K_2Cr_2O_7$ in distilled water & make 100 ml with distilled water.

2. Standard Chromium Solution :- Dilute 1 ml stock solution to 100 ml. i.e. 1 ml = 5.00 $\mu$g Cr

3. Sulphuric Acid :- 1:1 (i.e. 1 ml $H_2SO_4$ + 1 ml distilled water)

4. Phosphorbic Acid : Concentrated

5.Diphenyl Carbazide :- Dissolve 250 mg 1, 5 Diphenyl Carbazide in 50 ml acetone. Store in a brown bottle.

**PROCEDURE :-** Remove the color of the sample. Take 50 ml sample, add 1:1 Sulfuric Acid, 3 to 5 drops of Orthophosphoric acid Dilute it to 100 ml with distilled water then add 2 ml D.P.C. mix and let stand 5 to 10 minutes for full color development then take O.D.at 540 nm.
It is necessary to make a Standard Curve before the analysis of sample. Also run to the reagent blank.

**CALCULATION :-**

$$\text{Hexavalent Chromium } (Cr^{+6}) \text{ mg/l} = \frac{\text{O.D.} \times \text{Factor} \times 100}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** Hexavalent molybdenum and mercury salts, Vanadium iron, Ferric ion and Copper etc. are also interferences.

**PRECISION AND BIAS :-** The dissolved Chromium was determined in 31 Laboratories with synthetic sample containing 110 mg Cr/L, 500 mg Al/L, 50 mg Cd/L, 470 mg Ca/L, 300 mg Fe/L, 70 mg Pb/L, 120 Mn/L, 150 mg Ag/L and 650 mg Zn/L in the distilled water. The relative standard deviation was 47.8% and relative error was 16.3%.

## CHEMICAL OXYGEN DEMAND

**METHOD** :- Open Reflux Method - Followed by Titrimetric Method
Chemical Oxygen Demand test determines the oxygen required for chemical oxidation of organic matter with help of strong chemical oxidant.

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION :-** Conc. Sulfuric Acid

**APPARATUS :-** COD Digestion Apparatus

**REAGENTS:-**

a) Potassium DI- Chromate $k_2Cr_2O_7$ (0.25 N):- 12.259 gm dried $k_2Cr_2O_7$ in 1 liter distilled water.
b) Ferrous Ammonium Sulphate :- 0.1 N: 39.2 gm FAS + 20 ml conc. $H_2SO_4$ & make upto 1 liter with distilled water.
c) Ferroin Indicator Solution :- 1.485 gm 1.10 phenenthroline monohydrate + 0.695 gm $FeSO_4$ $7H_2O$ in 100 ml distilled water.
d) Potassium Hydrogen Phthalate (KHP) : 0.425 gm dried KHP in 1 liter distilled water.

**PROCEDURE :-** Take 10 ml Potassium dichromate in COD tube add 30 ml Conc. Sulfuric Acid containing $Ag_2SO_4$, add 1 gm Mercuric Sulphate and appropriate ml of sample (maximum 20 ml sample). Shake well the mixture and reflux for about 2 hours at 150 °C**.** Titrate against 0.1 N Ferrous Ammonium Sulphate using Ferroin indicator. Note down the burette reading. Also run the reagent blank.

**CALCULATION :-**

$$\text{Normality of FAS} = \frac{\text{N of potassium Dichromate} \times \text{Vol. of } K_2Cr_2O_7}{\text{ml of FAS used}}$$

$$\text{COD mg/l} = \frac{\text{Actual. Difference} \times \text{Factor}}{\text{Sample taken (ml)}} \qquad (\text{Factor} = \text{N of FAS} \times 8000)$$

**INTERFERENCE :-** Volatile straight – chain aliphatic compounds**,** Presence of halogens may interfere. Chloride, Bromide and Iodide ions. Nitrite ($NO_2$)

**HALIDE INTERFERENE REMOVAL :-** (A) By adding $HgSO_4$ .(B) By calculation – 1 mg Chloride contribute 0.23 mg COD. (C) Removal of Halide by adding $AgNO_3$ & $Ag_2SO_4$. (D) $NO_2$ by adding sulfamic acid in $K_2Cr_2O_7$.

**PRECISION AND BIAS :-** A set of synthetic samples containing KHP and NaCl was tested by 74 laboratories at a COD of 200 mg $O_2$/l in the absence of Cl, the standard deviation was $\pm$ 13 mg/l (Coefficient of variation 6.5%) at COD of 160 mg $O_2$/l and 100 mg Cl/l. The standard deviation was $\pm$ 14 mg/l (Coefficient of variation 10.8 %) .

## SULPHATE

**METHOD :-** Turbidimetric Method

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy

**PRESERVATION :-** Ice

**APPARATUS :-**

Conical flask, pipette
Magnetic stirrer
Spectrophotometer

**REAGENTS :-**

1. Conditioning Reagent:- 30 ml Conc. HCl + 300 ml distilled water + 100 ml 95% Ethanol or Isopropyl Alcohol + 75 gm NaCl + 50 gm Glycerol.

2. $BaCl_2$ powder or saturated solution of $BaCl_2$

3. Standard Sulphate Solution:- Dissolve 0.1509 gm anhydrous $Na_2SO_4$ in distilled water & dilute it to 1000 ml with distilled water.

**PROCEDURE :-** Take 50 ml Distilled water in the flask and add 5 ml Conditioning Reagent and one spatula $BaCl_2$ powder ( or 2 ml of saturated $BaCl_2$ solution ). Put the prepared flask on magnetic stirrer and add the sample until turbidity appears. Then add distilled water (Total volume of sample and distilled water must be equal to 50ml) & Measure turbidity on 420 nm.

It is necessary to make a Standard Curve before the analysis of samples. Also run the reagent blank.

**CALCULATION :-**

$$SO_4 \text{ MG/L} = \frac{\text{O.D.} \times \text{Factor} \times 100}{\text{Sample Taken (ml)}} \times \text{Dilution}$$

**INTERFERENCE :-** Silica in excess of 500 mg/l will interfere and large quantities of organic materials do not give satisfactory precipitation of $BaSO_4$.

**PRECISION AND BIAS :** With a turbidimeter in a single laboratory, with a sample having a mean of 7.45 –mg $SO_4$/L a standard deviation of 0.13 mg/L and a coefficient of variation of 1.7 were obtained. Two sample closed with sulfate gave recovery of 85% & 91%.

# CYANIDE

**METHOD :-** Colorimetric

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy or glass bottle.

**PRESERVATION :-** NaOH palates

**APPARATUS :-** Cyanide distillation unit, Spectrophotometer, Specific Ion meter.

**REAGENTS :-**

a) Sodium Hydroxide (1.25%) :- 50 gm NaOH in 1 liter distilled water (for distilation)
b) Magnesium Chloride :- 510 gm $MgCl_2$ in 1 liter distilled water.
c) Sulfuric Acid :- 1:1 (i.e. 1 ml Conc. $H_2SO_4$ + 1 ml distilled water)
d) Chloramine –T : 1 gm Chloramine – T in 100 ml distilled water.
e) Sodium Dihydrogenphosphate (1 M) :- 138 gm $NaH_2PO_4H_2O$ in 1 liter distilled water
f) Pyridine –Barbituricacid:- 15 gm Barbituricacid + 75 ml pyridine + 15 ml Conc. HCl & make upto 250 ml with distilled water.

**PROCEDURE :-**

1.**PRELIMINARY DISTILLATION METHOD :-** Take 100 ml preserved (in NaOH) sample in the boiling (250 ml round bottom) flask. Add 20 ml of $MgCl_2$ solution. Then add 50 ml of 1:1 $H_2SO_4$. Take 50 ml 1.25 N of NaOH solution. to the absorbing tubes. (Impingers 2 Nos. in series). Adjust suction so that approximately 1 air bubbles enters the boiling flask. This air rate will carry HCN gas from above flask to alkali absorber to convert in NaCN. Then heat the solution in the round bottom flask for one & half hour. After that mix all above impingers absorber soln. in 250 ml volumetric flask and make the volume 250 ml by D.W. Afterwards if CN is above 1 mg than analyse it by titrimetric method using rhodamine indicator against 0.0193 N $AgNO_3$ soln. If concentration is lower; then follow below mentioned colorimetric method or selective Ion electrode method.

**2. COLORIMETRIC METHOD :-** Cyanide in the alkaline distillate from preliminary treatment is converted to CNCl by reaction with Chloramine – T at pH less than 8 without hydrolyzing to CNO. After the reaction is complete CNCl forms red blue dye on addition of a pyridine Barbituric Acid. The absorbance is read at 578 nm.

Take distilled 50 ml sample and make volume 250 ml, from this take 50 ml diluted sample add 4 ml buffer soln. & 1 ml Chloramine – T soln. Let it stand for 2 minutes and add 4 ml of pyridine barbituric acid soln. Take O.D. at 578 nm, after 8 minutes and before 15 minutes.

**CALCULATION :-**

$$\text{CN mg/L} = \frac{\text{O.D.} \times \text{Factor} \times \text{Total vol. of abs. media}}{\text{Original volume of sample distilled}} \times \frac{1000 \text{ for ltr}}{\text{Taken for distillation}}$$

**INTERFERENCE :-** All known interference are eliminated or reduced to a minimum by Distillation.

**PRECISION AND BIAS :-** As a quality control measure, periodically test apparatus, reagents and other potential variable in the concentration range of interest. As an example at least 100 $\pm$4% recovery from 1 mg CN/l standard should be obtained ( Total CN after distillation ).

# FLUORIDE

**METHOD :-** Ion – Selective Method

The classical ion- selective electrode consists of a tube made of a good electrical insulator, which is closed at its lower end by a sensing membrane. Within the tube there is a filling solution known as internal filling solution containing a fixed column of the ion to which the membrane is sensitive.

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION :-** Ice

**APPARATUS :-**

1. Ion Selective Meter
2. Fluoride Electrode
3. Magnetic Stirrer

**REAGENTS :-**

For TISAB :- Take 500 ml distilled water + 170 gm $NaNO_3$ + 68 gm Sodium acetate trihydrate + 92.4 gm Tri Sodium Citrate and make the volume upto 1 litre with distilled water.

**PROCEDURE :-**

Adjust the pH of sample between 7 to 8 and take 20 ml of sample, add 20 ml of TISAB reagent. Put two appropriate range of standards. The slope should be between 50 to 60. Then analyze the sample. The instrument shows results directly in mg/l.

**CALCULATION :-**

Fluoride concentration can be measured in units of moles per liter, equivalents per liter, parts per million, or any convenient concentration unit.

**INTERFERENCE :-**

Fluoride forms complexes with several polyvalent cations notably aluminium and iron. In acid solution fluoride forms a poorly ionized HF. HF complex but the buffer maintains a pH above 5 so as to minimize hydrogen fluoride complex formation. In alkaline solution hydroxide ion also can interfere with electrode response to fluoride ion whenever the hydroxide ion concentration is greater than one tenth the concentration of fluoride ion.

**PRECISION AND BIAS :-**

A synthetic sample containing 0.850 mg/L, $F^-$ in distilled water was analysed in 111 laboratories by the electrode method, with a relative standard deviation of 3.6% and a relative error of 0.7%.

# BORON

**METHOD :-** Curcumin Method (Colorimetric Method)

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION :-** Ice

**APPARATUS :-**

a) Spectrophotometer
b) Porcelain evaporating dish
c) Water bath

**REAGENTS :-**

1. Curcumin Reagent :-
   Dissolve 40 mg Curcumin + 5 gm Oxalic Acid + 80ml Conc. HCL in 100 ml ethyl alcohol.

2. IPA (Isopropyl Alcohol)

**PROCEDURE :-** Take the sample in evaporating dish & add 4 ml Curcumin reagent - Evaporate in a water bath at 55° C For about 80 minutes. Dissolve the residue in 95 % Isopropyl alcohol and make up the volume 25 ml with it. Measure O.D. at 540 nm within 1 hour after red color is developed. Prepare a standard curve before analysis.

**CALCULATION :-**

$$\text{Boron mg/l} = \frac{\text{O.D.} \times \text{Factor}}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** NO3-N Concentration above 20 mg/l and Hardness above 100 mg/l will interference.

**PRECISION AND BIAS :-** A synthetic sample containing 240 Boron/L, 40 µg As/L, 250 µg Be/L, 20 µg Se/Land 6 µg V/L in distilled water was analyzed in 30 Laboratories by the Curcumin method with a relative standard deviation of 22.8 % and a relative error of 0%.

## MANGANESE (Mn)

**METHOD :-** Colorimetric (perssulfate method)

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy.

**PRESERVATION :-** Ice

**REAGENTS :-**

1. Special Reagent:- Dissolve 75 gm mercuric sulphate + 400 ml Conc. $HNO_3$ + 200 ml Distilled Water + 200 ml 85% Phosphoric Acid + 35 gm Silver nitrate & make up 1 litter with distilled water.

2. $H_2O_2$:- 2 drops per sample.

3. Ammonium Persulfate : 2 gm per sample.

**APPARATUS :-** Spectrophotometer, conical flask - 100 ml capacity, Nessler's tubes – 100 ml capacity.

**PROCEDURE :-** Take sample of 50 ml or 100 ml in conical flask. Add 5 ml Special reagent & 1 drops $H_2O_2$ and concentrated to 90 ml by boiling or dilute to 90 ml. Add 1 gm $(NH_4)_2S_2O_8$ bring to a boil and boil for 1 minute (do not heat on water bath), cool it, dilute it 100 ml after taking it in nessler tube and after reddish pink color is developed, Compare it with blank and take reading at 525 nm.
It is necessary to make a std. Curve before the analysis of sample. Also run the reagent blank.

**CALCULATION :-**

$$\text{Manganese } (Mn^{++}) \text{ mg/l} = \frac{\text{O.D.} \times \text{Factor}}{\text{Sample Taken (ml)}} \times \text{dilution}$$

**INTERFERENCE :-** Bromide and Iodide, Air interference makes Mn to $MnO_2$.

**PRECISION AND BIAS :-** A synthetic sample containing 120 µg Mn/L, 500 µg Al/l, 50 µg Cd/L, 110 µg Cr/L, 470 µg Cu/L, 300 µg Fe/L, 70 µg Pb/L, 150 µg Ag/L and 650 µg Zn/L. in distilled water was analyzed in 33 Laboratories by Persulfate method with relative standard deviation of 26.3% and a relative error of 0%. A second sample similar in all respect except for 50 µg Mn/l and 1000 µg Cu/L was analyzed in 17 Laboratories by same method with a relative standard deviation of 50.3% and relative error of 7.2%.

## AMMONICAL NITROGEN & TOTAL KJELDAHL NITROGEN

**METHOD :-** Distillation / Nasslerisation

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy.

**PRESERVATION :-** Conc. Sulfuric acid

**APPARATUS :-** Ammonia distillation apparatus

**REAGENTS :-**

1. Sodium Hydroxide (NaOH) 6 N :- 240 gm NaOH in 1 liter distilled water

2. Boric Acid Solution :- 20 gm $H_3BO_3$ in 1 liter Ammonia Free distilled water

3. Mixed Indicator Solution :- Dissolve 200 mg (0.2 gm) methyl red indicator in 100 ml 95% ethyl or isopropyl alcohol. Dissolve 100 mg (0.1 gm) methylene blue in 50 ml 95% ethyl or ethyl alcohol, combine above two indicator solutions. Prepare monthly.

4. 0.1 N $H_2SO_4$ (Stock) : Take 2.8 ml $H_2SO_4$ in 1 liter distilled water.

5. 0.02 N $H_2SO_4$ :- Take 200 ml stock solution & make up 1000 ml with distilled water.

6. 0.02 N $Na_2CO_3$ :- Take 1.065 gm $Na_2CO_3$ → dissolve in 1 liter distilled water to standardize with 0.02 N $H_2SO_4$

**PROCEDURE :-**

FOR $NH_3$-N :-

Take 50 ml Sample. Add 2 drops phenopthalein indicator and 6N NaOH – distilled in Boric acid added with mix indicator. If ammonia is present Boric acid will be convered into blue to green. Titrate against 0.02N $H_2SO_4$ till the color change from green to blue.

FOR T.K.N.:-

Take appropriate volume of water sample. Add 10 ml sulfuric acid, add 5 gm $CuSO_4$, 5 gm $K_2SO_4$ and digest upto 370° C for @ 2 hours and follow procedure as per $NH_3$- N.

FOR NESSLERISATION:-

Take 50 ml sample and 1 ml Nessler reagent. Wait for 10 minute until colorless to yellow color is developed and take O.D. at 425 nm. It is necessary to make a Standard Curve before the analysis of sample. Also run the reagent blank.

**CALCULATION :-**

$$\text{For } NH_3\text{- N mg/l \& T.K.N.} = \frac{\text{B.R.} \times 14 \times 0.02 \times 1000}{\text{Sample Taken (ml)}}$$

$$\text{For Nesslerisation method } NH_3\text{- N} = \frac{\text{O.D.} \times \text{Factor}}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** Glycine urea, Glutamic acid and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7 % at this pH for urea and about 5% for cynates. Volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Residual chloride reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with dechlorinating agent. (Dissolve 3.5 gm Sodium Thiosulfate in 1000 ml distilled water.)

**PRECISION AND BIAS :-** Synthetic samples containing ammonia and other constituents dissolved in distilled water were analysed by titration method.

Sample 1 contained 200 $\mu$g $NH_3$- N/L, 10 $\mu$g Cl/L, 1 $\mu$g $NO_3$- N/L, 1.5 $\mu$g organic – N/L, 10 $\mu$g $PO_4$/L and 5 $\mu$g silica/L. The relative std. Deviation and relative error for the 21 participating Laboratories were 69.8% and 20% respectively.

Sample 2 contained 800 $\mu$g $NH_3$- N/L, 200 $\mu$g $Cl^-$/L, 1.0 mg $NO_3$-N/L, 0.8 $\mu$g organic N/L, 0.5 $\mu$g $PO_4^{3}$/L, and 15.0 $\mu$g silica /L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5% respectively.

Sample 3 contained 1500 $\mu$g $NH_3$- N/L, 400 $\mu$g Cl/L, 1.0 $\mu$g $NO_3$- N/L, 0.2 $\mu$g organic N/L, 0.5 $\mu$g $PO_4^{3}$/L, and 30.0 $\mu$g silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6% and 2.6% respectively.

## NITRATE – $NO_3$-N

**METHOD :-** Colorimetric Method (Phenol Di-Sulphonic Acid Method)

**COLLECTION OF SAMPLE :-** Collect the sample in plastic carbouy.

**PRESERVATION :-** Ice

**APPARATUS :-**

a) Beaker – 100ml cap.
b) Nessler Tube – 100-ml cap
c) Hot air oven
d) Spectrophotometer

**REAGENTS :-**

a) P.D.A.(Phenol Disulphonic Acid)
b) Ammonia Solution

**PROCEDURE :-** If necessary decolorize the sample and take 50 ml sample in 100 ml capacity beaker and dry at 105°C in oven for 12 hrs. After cooling add 2 ml PDA solution and rub the residue thoroughly. Dilute with 20ml distilled water. Transfer in 100 ml. Nesseler tube then add 10 ml Liquor Ammonia solution and make up final volume 100 ml with distilled water. Measure O.D. at 410nm.
It is necessary to make a std. Curve before the analysis of sample. Also run the reagent blank.

**CALCULATION :-**

$$NO_3\text{-N mg/l} = \frac{\text{O.D.} \times \text{Factor}}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** Chlorides interfere seriously with the determination of nitrates because of their recycling action.

**PRECISION AND BIAS :-** Accuracy of the order of $\pm$ 0.1 mg/l $NO_3$-N can be obtained only by the proper treatment of the chloride and nitrite interference.

A synthetic unknown sample containing 1 mg/L $NO_3$-N, 10 mg/L Cl, 200 mg/L $NH_3$-N, 1.5 mg/L Organic-N, 10 mg/L $PO_4$ and 5 mg/L silica in the distilled water was determined by phenoldisulfonic method with a relative standard of 74.4% and relative error of 38% in 46 Laboratories. A second synthetic unknown sample containing 1 mg/L, Nitrate – N, 200 mg/L, Cl, 800 mg/L $NH_3$-N, 800 mg/L organic-N, 5 mg $PO_4$ and 15 mg/L, silica in distilled water was determined by the Phenoldisulfonic acid method with a relative standard deviation of 57.9% and a relative error of 31% in 46 Laboratories.

## PHOSPHATE

**METHOD :-** Stannous Chloride Method

**COLLECTION OF SAMPLE :-** Collect the sample in the plastic carbouy

**PRESERVATION :-** Ice

**APPARATUS :-** Spectrophotometer

**REAGENTS :-**

1. Strong Acid :- 300ml. Conc. $H_2SO_4$ 600 ml Distilled water. 4 ml Conc. $HNO_3$. Make up 1 liter with distilled water.

2. Ammonium Molyblate : 25 gm Ammonium Molyblate + 175 ml distilled water + 280 ml Conc. $H_2SO_4$ + 400 ml Distilled water & make up 1 liter with distilled water.

3. Stannous Chloride :- Dissolve 2.5 gm $SnCl_2$ $2H_2O$ → 100 ml Glycerol.

**PROCEDURE :-** Take 100 ml of sample in conical flask. Add 4 ml strong acid & digest it on hot plate upto 15 to 20 ml and cool it. Then maske volume to 100 ml in nesseler tube with distilled water and add 4 ml ammonium molybdate and add 10 drops $SnCl_2$ Now put it 10 minutes for color development. Blue color will obtain from colourless. It $PO_4$ is present measure O.D. at 690 nm

It is necessary to make a Standard Curve before the analysis of sample. Also run the reagent blank.

**CALCULATION :-**

$$PO_4 \text{ mg/l} = \frac{\text{O.D.} \times \text{Factor}}{\text{Sample Taken (ml)}}$$

**INTERFERENCE :-** Silica and Arsenate cause positive interference. Only if the sample is heated Arsenite Flouride, Thorium, Bismuth, Sulfide, and Thiosulphatethiocynate or excess molybdate cause negative interferences.

**PRECISION AND BIAS :-** The minimum detectable concentration is about 3 $\mu$g /L. The sensitivity at 0.3010 absorbance is about 10 $\mu$g/L for an absorbance change of 0.009.

## HEAVY METALS (Cu, Pb, Ni, Zn, Fe, Total Cr, Cd, Hg,).

There are several methods available for analysis of trace Metals Heavy Metals covering both Chemicals and Instrumental Methods. Some of them are as under:

1. Colourimetry.
2. Atomic Emission Spectrometry.
3. Atomic Absorption Spectrometry.
4. U.V. Spectrometry.
5. Inductive Couple Plasma Atomic Absorption Spectrometry.
6. Infrared Spectrometry.
7. Electro-Chemical Method (Electrometry)
8. Neutron activation
9. Fluorimetry.

The above analytical methods are being used for the metal – trace metal analysis in the field of Environmental for Water, Waste water, air, solid waste - hazardous waste etc.

### 1. SAMPLE COLLECTION, PRESERVATION AND PREPARATION

One of the most important part if trace metal-metal analysis is the sample collection, preservation and preparation of the sample

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

The sample should be handled in such a way that no significant changes in the composition occur before the tests are made.

**General Requirement:**

Obtain a sample that meets the requirements of sampling programme and handle it so that is does not deteriorate or become contaminated before it is analysed.

Ensure that all sampling equipments ( containers- bottles, buckets, rnug, funnel etc.) is clean and quality assured before use.

Use sample container that are clean and free of contaminants and also should be free from the contamination of the metals to be analysed.

Fill the samples in cleaned and well washed containers before that rinse the sample container.

Moreover one important factor which effect the analysis is that you have to clean your sample container and the glassware with the tape water, neutral detergent, distilled water and with the dilute Nitric Acid.

Prior to sampling it is essential to rinse the sample containers and other material to be used for the sample collection should be rinsed with the 1+1 $HNO_3$

Generally trace-metal samples except mercury should be collected in the plastic good quality, PE-Ploy Ethylene containers. The plastic material should not react with sample of metals i.e with the analyte (the metal to be analysed) which are to be analyse.

For General metals-Minimum sample size-volume should be 1000 ml should be collected.

To analyse the dissolved metals is the sample of water and waste water sample should be filtered immediately.

For Chromium VI the sample should be collected in the same type of container but the volume of the sample to be collected is recommended is 100 ml and maximum storage period is 24 hours. For Mercury follow the same procedure for sample collection as described for general other metal moreover, one important factor which effect the analysis is that you have to clean your sample container and the glassware with the tape water, neutral detergent , distilled water and with the dilute Nitric Acid.

All the samples for which the metal analysis is to carried out should be preserved with Con. $HNo_3$ (Nitric Acid) to pH <2

By preserving the sample you can minimize the precipitation and adsorption of the metals on the container's wall.

Generally 1.5 ml/Lit. Conc. $HNO_3$ or 3 ml/Lit. 1+1 $HNO_3$ is to be used for the preservation of the sample for metal analysis.
After the acidification of the homogenised sample preferably should stored in a refrigerator at approx. 4 $^0$C to prevent changes in volume due to evaporation.

To minimize the potential for volatization or bio degradation between sampling and analysis keep samples as cool as possible

The best sample containers are made up of quartz or TFE. These containers are very expensive; it is preferred to collect the samples in PE polyethylene container.

## 2. PRELIMINARY TREATMENT OF THE SAMPLE:( Sample Preparation)

Samples containing organic or particulate material, generally required pre-treatment before analysis "Total Metals" includes all metals, inorganically and organically bound, both dissolved and particulate.

Colorless, transparent samples, turbidity of 1 NTU no odour and single phase may be analysed directly by Atomic Absorption spectrometry or other method without digestion.

If the dissolved or suspended metals are to be determined, filter the sample at the time of collection.

Extractable metals are lightly absorbed on particulate material, Because some sample digestion may be unavoidable use rigidly controlled condition to obtain meaningful and reproducible results. Maintain constant sample volume and contact time. Express results as extractable metals and specify extraction. During preliminary treatment of samples care should be taken so that introduction of other metal cannot take place.

Reagents, acids and chemicals which are used in the analysis should be of ultra pure quality.

The distilled water to be used for the analysis should be metal free deionised water.

## 3. PRELIMINARY DIGESTION OF THE SAMPLE FOR METALS:

- Digestion for metal sample is quite necessary to remove organics and to get free metal ions; also to get dissolve the metals and can concentrate the metal in the sample if it is in very less quantity.
- With the help of digestion you can reduce the remaining oxidising agents and also you can reduce the interferences of the organic matter.
- By digestion you can do pH adjustment.
- Sometime it is essential and useful to use buffer solution plus indicator. Add buffer solution and indicator to the sample solution in normal extraction procedure it should be carried out at 3 to 3.5 pH.
- Separation of metals can be done and also you can enrich the metals

Separation of Metal extraction can be also done with the help of digestion- Microseparator, separating funnel, volumetric flask are used.

**<u>Requirements for Digestion & Extraction</u>**

1. Erlenmeyer-conical flask 250 ml cap. Or digestion tubes, measuring cylinders 25.50, 100 ml cap. Fynnel.
2. Dispensers, Pipettes, Micropipettes.
3. Digestion Unit/Heating Block or Hot plate.
4. Con. $HNO_3$, $H_2SO_4$, $HclO_4$, $H_2O_2$, $KMNO_4$, $K_2S_2O_8$, HCl,

5. Good quality of distilled water / Double distilled water.
6. 50,100 ml cap. Volumetric Flask, filter paper
7. Beakers, Hand-gloves Acid Alkali proof, Asbestos Hand gloves, Safety goggles.

And other required material.

Always prepare an acid blank for each type of digestion performed.

Experience indicates that a blank made with the same acids and subjected to the same digestion procedure as the sample can correct for impurities present in acids, in reagent water. Here some digestion procedures are given.

NOTE: Always make up the same volume of the digested sample for which you have taken for digestion (before digestion).

### I. Nitric Acid Digestion:

Mix well (homogenised) sample and transfer a suitable volume (50 to 100 ml) to a 250 ml conical flask or in a digestion tube). Add 5 ml Conc. $HNO_3$ and few boiling chips, or glass beads. Bring to a slow boiling and evaporate on a hot plate or on the digestion block- Digestion unit to the lowest possible volume (about 10 to 20 ml) before precipitation occur. Continue heating and adding nitric acid (Con $HNO_3$) as necessary until digestion complete. Do not let sample dry during digestion.

Wash down conical flask or digestion tubes (which used for the digestion) well with distilled water then filter if necessary. Transfer the filtrate to a volumetric flask of 50 or 100 ml cap. Rinse the digestion vessel and make up the volume to the make with distilled water. Mix thoroughly.

Take the portion of the digested sample for the analysis of directly can be used for the determination of metal in atomic Absorption spectrophotometric analysis.

Here we use Con. $HNO_3$ Nitric Acid for digestion because it is a strong oxidizing agent and most of the metals are soluble in it.

### II. NITRIC ACID-HYDROCHLORIC ACID DIGESTION:

Transfer a measured (50 or 100 ml) volume of well mixed homogenised and acid preserved sample in to a conical flask 250 ml cap, or in digestion vessel-tubes. Add 3 ml Con. $HNO_3$ evaporate it on a hot plate or on heating block or on a digestion unit, add 1+1 HCL and heat again the sample to dissolve any precipitate or residue. Give distilled water wash and filter if necessary to a 100 ml volumetric flask and make up the volume up to the mark.

Use this digested sample for analysis.

## ATOMIC ABSORPTION SPECTROPHOTOMETER

Scientist Walsh, Alkemade, and finally Winefardener mainly contributed for the establishment and demonstration in the year 1962 for the trace metal analysis.

Now a days Atomic Absorption Spectrophotometer has become one of the most extensively employed technique for determination of trace metals. It is a high attainable sensitivity for a wide range of element, and a high sensitivity for the analyte element which is to be analyse.

Atomic Absorption Spectroscopy is the term used when the radiation absorb by atoms is measured. Atomic Absorption is a part of Spectroscopy. AAS- Atomic Absorption Spectrophotometer follows the Lambert & Beer's law like other Spectrometer of Spectroscopy.

Like the Flame Photometer AAS consists of a Flame, a Grating, a Monochromater to isolate emission line. Detector and an Amplifier. In addition to that the absorption system is a light source – Hollow Cathode Lamp of Electron Discharge Lamp which emits a stable and intense light particular wavelength.

Each element has characteristic wave length. A radiation – light source with wave length readily absorbed by the element to be determined is directed through the flame and measure of it's intensity of the sample introduced in the Flame. The Decrease in the intensity of the light observed with the sample is a measure of the concentration of the element.

## PRINCIPLE

The AAS method for metal analysis is based on the fact that atoms in their ground state can absorb light of a particular energy (frequency). In AAS light of a different wavelength radiates through the atomizer system ( i.e. flame or graphite furnace- cuvette) and is absorb by atoms in the ground stats. The quantity of absorbed light is proportional to the concentration of non-excited atoms (ground state atoms). It is measured as resonance in a detector.

Light is emitted at the source of radiation and absorbed by atoms in the atomizer system at exactly defined wavelength and within strictly limited spectral ranges. Each spectrum line is specific for different element. As every element has it's own characteristic wavelength (which is indicated to the absorption wavelength in the ground state)

## TYPES OF TECHNIQUES FOR ATOMIC ABSORPRION SPECTROMETRY

Following techniques are generally being used for the analysis of Metal analysis.

- Flame Atomic Absorption Spectrophotometry- Flame AAS
- Graphite Tube- Furnace AAS
- Vapour Generation AAS,Hydride AAS, Cold Vapour

**Flame AAS**

In flame AAS you can determine –analyse the concentration of metal in the mg/l or ppm level. Here Air – Acetylene Flame, $N_2O$- acetylene flame is being used Generally Air Acetylene flame is widely being used.

Air is used as an Oxidant or fuel supporting gas. As a fuel Acetylene or Nitrous Oxide is used. Both the gases as well as Air should be of good quality i.e. it should be interferences free. At the time of operating certain pressure is required of the gases or Air. Mixture of Air Acetylene and Nitrous oxide Acetylene is generally used. Temperature of Air Acetylene is less than the temperature of $N_2$- Acetylene flame. Nitrous Oxide – Acetylene is used when the oxides of elements like Mo. Be, and V are thermally too stable to be dissolve in the Air Acetylene flame. The temperature can be reached up to $3150^0$ in the $N_2O$- Acetylene flame where as in Air – Acetylene flame the temperature will reach up to $2500^0$C Different burners are used for Air Acetylene and Nitrous Oxide- Acetylene flame.

**Nebulization-Atomisation:**

To atomize the liquid sample in a flame AAS the sample is sprayed via a pneumatic sprayer into a mixing chamber where it is mixed with a combustible gas (Acetylene) and Oxidant Air or Nitrous Oxide. Then reaches to the flame through Burner. As a result of the heat dissociation takes place in the Atoms. These atoms absorbs the light radiation at a define wavelength from the Hollow Cathode Lamp- HCL. Sample introduction should also be maintain to have better atomization and also you will get fine size of aerosol for atomisation. Monochromator/gratting is isolating the emission lines of the element. The isolated light is directed on the detector. This is a Photo-multiplier Tube (PMT) which produces electric current which is depends on the intensity of the light. The current is then amplified and processed by a electronic system to produce a signal which is a measure of light attenuation occurring in the sample cell. This signal can be further processed to produce on instrument readout directly in the concentration units.

**GRPHITE FURNACE AAS:**

In this AAS technique Graphite furnace and graphite tube is used. The generation of atoms by means of an electrically heated graphite furnace atomizer. This technique is used for measuring elements metals concentrations in ppb- Parts Per Billion level with better accuracy. Graphite furnace AAS contributed by L'vov and Marzmann who had developed this technique.
In graphite furnace , HCL Hollow cathode Lamps, graphite tubes (Pyrolytic-coated and non coated) platform furnace are being used.

It is also a part of AAS but for the analysis of trace metals in ppb conc. Where Flame AAS can not be advisable to use, it is applicable. In this graphite furnace AAS, furnace has a very high temperature to generate a population of free atoms so that the atomic absorption can be measured. This is generally achieved in three stages.

- A Drying
- An Ashing and
- Atomization

Inert gas like Nitrogen/Argon is used to protect the incent-decent graphite from excessive corrosion. Here is a upward flow of $N_2$/Ar which surrounds the graphite- heater grahite. $N_2$/Ar gas also sweeps away any product from the light path.

Compare to Flame and Vapour generation – Hydride generation techniques here in the Graphite Tube AAS very small quantity of sample is required and the measurement can be done in ppb level. The graphite AAS is 100 times more sensitive than flame. Sample volume ineeded is 5 to 100 microliter.

## VAPOUR GENERATION/ HYDRIDE GENERATION SYSTEM-AAS

It is also one of the techniques of AAS. Some elements which generates their hydride and cold vapour , are measured by this method. As , Se, Bi, Te, Sn, Sb, and Hg are the elements which can be measured. In acidic condition above elements produced their hydrides with reducing agents $NaBH_4$ – Sodium Boro Hydride. Then it is treated in the absorption cell which is specially made of Silica glass. Then atomic absorption is measured. In cold vapour generation AA system reagents are the same but it's concentration is different than the Hydride generation. Mercury can be measured with this technique. Mercury can be brought in to vapour phase without flame or heating or temperature. Due to it's sensitivity flame methods are not used. Chemical reduction method is used. Mercury ions are reduced to metallic mercury with $NaBH_4$ & HCL with the inert gas $N_2$.The cold vapour of mercury swept in the absorption cell from which the radiation passed and measures the absorption. Finally we can get the concentration.

## SAFETY & MAINTENANCE OF THE INSTRUMENT:

- Here are some the important points for safety.
- Along with the Analysis you have to take extreme care of your safety and the safety of the instrument.
- Always operate the instrument at required voltage.
- Always use the correct burner and gas regulators.
- Drain the Air tank of the compressor (if provided) intermittently.
- Replace-change the Acetylene gas cylinder when the min. pressure is left (mentioned in the instruction manual of the instrument supplier).
- In the case of Varian Spectra AA-20 min at a pressure of Acetylene 7 kg/$Cm_2$ never inst. Can be operated. Otherwise there will be a chance of an accident.
- Always use safety wares while analysing on AAS.
- Never keep the flame of AAS un attainable.
- Check Exhaust system always. Check the pipes and as pressure regularly.
- Inst. Should be serviced and clean regularly. Routine maintenance should be done periodically.

# SILICATE IN SEAWATER

The element silicon is the most abundant element in the universe. During weathering of rocks, silicate is brought into solution and thus is present in sea water. The average concentration is around 1 mg/l, much below its saturation value of 50 mg/l. In addition silica is also present in particulate, in varying quantities.

Many believe that the distribution of silica in sea water is controlled by processes involving organisms. Thus, hydrated silica is a major constituent of diatoms, which form a large proportion of phytoplankton. When the organisms die, silicon is liberated. The element passes through these cycles many times, in one season. It is estimated that approximately 120 million tonnes of silicon is removed from sea per annum by the growth and sedimentation of phytoplankton.

**DETERMINATION OF REACTIVE SILICATE**: The determination is based on the formation of a yellow silicomolybdic acid when an acidified solution of the sea water is treated with molybdate. This complex exists in two isomeric forms, depending on pH, which differ in their hydration. The α isomer is formed at pH 3.5 – 4.5 and is very stable, once formed. On the other hand, the β form is rapidly formed in the pH range 0.8 – 2.5, but it is much less stable. However, the latter has a higher molar absorptive.

Since both the isomeric forms have only low intensity absorbance, several methods have been developed to reduce the complexes to intensely coloured blue complexes. For the purpose, several organic and inorganic reducing agents have been used. In the present method, the use of oxalic acid has been recommended.

**SENSITIVITY :-** The molar absorptive is around 19,000 in sea water, lower than that in distilled water of 22,000.

**PRINCIPLE**: The seawater sample is allowed to react with molybdate under conditions which result in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complexes. A reducing solution, containing metol and oxalic acid, is then added which reduces the silicomolybdate complex to give a blue reduction compound and simultaneously decomposes the phosphomolybdate or arsenomolybdate eliminates the phosphate and arsenate interference.

**REAGENTS :-**

1. **Molybdate reagent:** Dissolve 4 gm of ammonium paramolybdate ($NH_4Mo_7O_{24}.4H_2O$) in about 300 ml of MQ water and add 12.0 ml of conc.HCl, mix well and make the volume to 500 ml with MQ water. Store it in a polythene bottle.

2. **Metol – Sulphate solution:** Dissolve 1.5gm of anhydrous sodium sulphite ($Na_2SO_3$), in 125ml of MQ water and then add 2.5gm of metal (p-methylaminophenol sulphate). Store in a plastic bottle.

3. **Oxalic acid solution:** Prepare a saturated oxalic acid solution by shaking 10g of oxalic acid dehydrate $(COOH)_2$, $2H_2O$ (AR) with 100 ml of MQ water; decant the solution from the crystals for use.
4. **Sulphuric acid solution (50%V/V):** Add slowly 100ml of conc. $H_2SO_4$ into 250 ml of MQ water and allow to cool.

5. **Reducing reagent:** Mix 100 ml of metol - sulphate solution with 60 ml of oxalic acid solution. Add 60 ml of 50% sulphuric acid solution slowly with mixing and make the mixture to 300 ml with MQ water.

6. **Preparation of synthetic sea water:** Add 25 g NaCl + 8 g $MgSO_4.7H_2O$ in 1000 ml MQ water.

**APPARATUS :-** Spectrophotometer: With 1 cm path length cell. Plastic containers: With marking at 25 ml volume. Standard flasks: 100ml and 25ml. Standard pipettes: 5ml and 10ml.

**PROCEDURE :-**

**Preparation of standard solution**

1. Dissolve 0.0188 g of sodium Silicofluoride ($Na_2SiF_6$) in 100 ml of synthetic seawater and store this stock solution in a polythene container. This solution contains 1000 µmol Si/L.
2. Prepare 5, 10, 15, 20 and 25 µmol Si/L by transfer 0.5, 1, 1.5, 2.0 and 2.5 ml of the stock solution and make up the volume up to 100ml with same synthetic seawater. These solutions contain 5, 10, 15, 20 and 25 µmol Si/L concentrations.

0.0188 g in 100 ml = 1000 µmol Si /L

1 ml from 1000 µmol solution in 100 ml = 1 µmol Si /L.

5 ml from 1000 µmol solution in 100 ml = 5 µmol Si /L.

10 ml from 1000 µmol solution in 100ml = 10 µmol Si /L.

15 ml from 1000 µmol solution in 100ml = 15µmol Si /L.

**Calibration of standards and blank**: Measure out 25 ml of MQ water for blank in triplicate. Similarly measure out 25 ml of working standard solutions in clean plastic container in triplicate. Add 10 ml of molybdate solution to each tube mix well and allow to stand for 10 min, add 15 ml of reducing reagent rapidly and mix immediately. After 2 hours incubation measure the absorbance of blank A(b) and standard solutions A (st) in a spectrophotometer using 1 cm cell at 810 nm against MQ water as reference.

**Sample analysis:** Measure out 25ml of the sample (triplicate) in a clean plastic container and add 10 ml of molybdate solution to each tube, mix well and allow to stand for 10 min, then add 15 ml of reducing reagent and mix immediately. Measure the absorbance A (s) of the sample in 1 cm cell at 810 nm.

## CALCULATION :-

**Calculation for Factor value (F):**

$$\mathbf{F} = \frac{\text{Conc. of standard solution}}{\text{A(st) - A(b)}}$$

Where A (st) = Mean absorbance of standards.

A (b) = Mean absorbance of blanks.

Calculation the amount of reactive silicate present in the sample

**Silicate – Si µmol/L** = F x A (s) – A (b)

Where A (s) = Mean absorbance of samples.

A (b) = Mean absorbance of blanks.

# SALINITY

**METHOD :-** Mohr-Knudson argentometric titration method.

Chlorinity: Chlorinity is defined as the mass in grams of pure silver necessary to precipitate the halogens in 0.3285255 kg of seawater (All weights are vacuum weights).

Salinity: Salinity is defined as the weight in grams (in vacuo) of solids that can be obtained from 1 kg of seawater (also measured in vacuo), when all of the carbonate has been converted to oxide, the bromine and iodine replaced by chloride, all organic matter oxidized, and the residue dried at 480°C to constant weight.

**Outline of the method**

Standard solution of silver nitrate is used to precipitate halide ions in seawater using potassium chromate as an indicator, to form silver halides. When a slight excess of silver ions are present, red silver chromate is formed.

**REAGENTS :-**

1. Standard solution of seawater (SSW): Use known chlorinity ($19.375\times10^{-3}$) / salinity (34.99 ppt) or as quoted for seawater (SSW) supplied by the institute of oceanographic science in Wormley, Godalming, Surray, (U.K.) in sealed glass ampules for standardizing silver nitrate solution.

2. Silver nitrate solution: Dissolve 25 g silver nitrate (AR) in1000 ml DW. Store in an amber glass bottle.

3. Potassium chromate solution: Dissolve 8 g potassium chromate (AR) in 100 ml DW. Store in a stoppered glass bottle.

**APPARATUS :-**

1. Burette: 25 ml, accuracy 0.1 ml
2. Bulb pipette: 5 ml, Accuracy 0.1 ml
3. Conical flask: 50 ml
4. Magnetic stirrer
5. Magnetic needle

**PROCEDURE :-**

**Standardisation of silver nitrate solution** : Pipette out 5.0 ml SSW into a clean conical flask, add 6 drops of potassium chromate indicator and titrate with silver nitrate solution from the burette while stirring vigorously on a magnetic stirrer. Clean the inner wall of the

flask with a jet of distilled water frequently and continue the titration. When colour change is observed, slow down the addition of titrant to drop by drop till colour change is observed from yellow to dirty orange. Repeat the standardization at least thrice and find out the mean of burette readings [BR (SSW)]. Find out the standardization factor F as follows:

$$F = \frac{\text{Chlorinity of SSW}}{\text{Mean BR (SSW) (ml)}}$$

**SAMPLE ANALYSIS :-**

Pipette out 5.0 ml sea water sample into a clean conical flask. Add distilled water (25 ml along the wall of the flask). Add 6 drops of indicator. Titrate against silver nitrate in the same manner as described as above. Obtain the reading (ml) [BR(s)].

**CALCULATIONS :-**

Calculate the "normalised volume" (V) from the Equation

$$V = BR(s) \times F$$

Obtain the correction factor (k) corresponding to V from established table and then calculate the chlorinity (Cl) and salinity (S) by using the relations

$$Cl = V + k$$

$$S = 1.80655 \times Cl$$

If salinity of SSW is given, calculate salinity of samples as follows:

$$F = \frac{\text{Salinity of SSW}}{\text{Mean BR (SSW) (ml)}}$$

$$\text{Salinity (ppt)} = F \times BR(s) \text{ (ml)}$$

**Note:** The salinity can be measured *insitu* by CTD probes/ dedicated salinometer / hand held refractometer etc. However, the argentometric, titrimetric procedures can be employed for finding out the instrumental error if any. The titrimetric procedure can be improved by using autotitrators.

# TOTAL COLIFORM & FAECAL COLIFORM

**METHOD :-** Multiple tube fermentation technique

**COLLECTION OF SAMPLE :-** Collect the sample in sterile glass bottle.

**PRESERVATION :-** Ice

**APPARATUS :-** Autoclave, Incubator controlled at 37°C and 44°C Gas burner, Nichrome wire loop (diameter of 3 mm), Sterile pipettes and sterile dilution water bottles.

**REAGENTS :-**

a) Mac conkey's broth. Single & Double strength
b) Brillient green bile lactose broth
c) Covav's reagent and peptone water

**PROCEDURE :-** Inoculate 10 ml of sample in three double strength (each of 10 ml) fermentation tubes, add 1 ml of sample in three single strength (each of 5 ml) fermentation tubes and 0.1 ml in three single strength (each of 5 ml) fermentation tube near the flame. Then incubate at 37°C for 24 hours and note down the positive tubes showing acid or gas Production. Reincubate the tubes which shows negative results.

**CONFIRM TEST:-** Transfer 1-2 loopful of culture from the presumptive Positive tube in each of two brilliant green broth each of 5 ml fermentation tube and incubate One tube at 37°C for 24 hr and other tubes at 44°C for 24 hr. The formentation of gas in the tube after 24 hr, 48 hr. shows positive test. Also inoculate 1-2 loopful of culture in peptone water tube (each of 5 ml) and incubate at 44°C for 24 hours.

**CALCULATION :-** Find out the MPN/100ml by the help of MPN index. Tubes which are Showing positive (gas formation) result at 37°C are Coliform organism and positive at 44°C are Fecal Coliform organisms. While addition of Covav's reagent in peptone water tube, the red color is developed it indicates positive result.

**INTERFERENCE :-** To avoid contamination procedure should be done in sterile condition.

**PRECISION AND BIAS :-** Not Applicable

# ANALYTICAL METHOD REFERENCE LIST

| Sr. No. | PARAMETER | TEST METHOD / STANDARD AGAINST WHICH TESTS ARE PERFORMED |
|---|---|---|
| 1 | Colour | [IS: 3025 (Part – 4) – 1983 (Reaffirmed 2002) Pt-co. Method Visual Comparison Method] |
| 2 | Temperature $^{o}C$ | ICMAM Protocol Method |
| 3 | Turbidity. | Nephelometric method. (2130 B APHA Standard Methods 21st Edition.) |
| 4 | pH Value | Electrometric Method IS: 3025 (Part – 11) – 1983 (Reaffirmed 2002) |
| 5 | Conductivity | 2510 B APHA Standard Methods 21st Edition. |
| 6 | Suspended Solids | Gravimetric method. (2540 D APHA Standard Methods 21st Edition.) |
| 7 | Total Dissolved Solids | Gravimetric method. (2540 C APHA Standard Methods 21st Edition.) |
| 8 | Chloride | Argentometric method. (4500 Cl— B APHA Standard Methods 21st Edition.) |
| 9 | Dissolved $O_2$ | Winkler method – Azide modification. (4500-O – C APHA Standard Methods 21st Edition.) |
| 10 | BOD | 3 – day BOD test. (IS 3025 (Part 44) 1993 Reaffirmed 1999) |
| 11 | Oil & Grease | Liquid – Liquid Partition Gravimetric method. (5520 B APHA Standard Methods 21st Edition.) |
| 12 | Phenolic Compound | 4 Amino Antipyrene method without Chloroform Extraction (Direct Photometric method) (5530 D APHA Standard Methods 21st Edition.) |
| 13 | Hexavalent Chromium | Colorimetric method APHA (21st Edition) – 3500 – Cr B : |
| 14 | COD | APHA (21st Edition)- 5220 B Open Reflux Method |
| 15 | Sulphate | Turbidimetric method APHA(21st Edition) 4500 $SO_4$ E |
| 16 | Cyanide | Preliminary Distillation treatment followed by Colorimetric method. (4500 - CN— E APHA Standard Methods 21st Edition.) |
| 17 | Fluoride. | Ion Selective Electrode method. (4500 - F— C APHA Standard Methods 21st Edition.) |
| 18 | Boron | Colorimetric Curcumin method. (4500-B B. APHA Standard Methods 21st Edition.) |
| 19 | Manganese | Colorimetric ( Persulfate Method ) (3500 – Mn B. APHA Standard Methods 21st Edition.) |

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|---|---|---|
| 20 | Ammonia Nitrogen. | 1).Titrimetric method<br>2).Nesslerization method. (4500 $NH_3$ B & C APHA Standard Methods 18st Edition. And IS: 3025 [Part 34]-1988 Method) |
| 21 | Nitrate Nitrogen. | 1)Spectrophotometric method. (213 D APHA Standard Methods 13th Edition.) **OR**<br>2) Spectrophotometric (Cadmium Reduction method 4500 – NO3 - E APHA standard methods 21st Edition.) |
| 22 | Phosphate | Stannous Chloride method. (4500 – P D APHA Standard Methods 21st Edition.) |
| 23 | Heavy Metals<br>(Cu, Pb, Ni, Zn, Fe, Total Cr, Cd, Hg,). | Flame Atomic Absorption Spectrometr. (3111 B APHA Standard Methods 21st Edition.) |
| 24 | Reactive silicate | ICMAM Protocol Method |
| 25 | Salinity | ICMAM Protocol Method |
| 26 | Total Coliform<br>Faecal Coliform | Multiple Tube Fermentation (MTF)Method |

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