GUIDANCE MANUAL

FOR

DRINKING WATER QUALITY MONITORING

GOVERNMENT OF ANDHRA PRADESH

RURAL WATER SUPPLY & SANITATION DEPARTMENT

VIJAYAWADA

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Foreword

The Rural Water Supply & Sanitation Dept., Govt.of A.P., has been making efforts to ensure the supply of treated and safe drinking water to the rural population especially in saline affected coastal habitations and Fluoride Affected habitations. Providing the safe drinking water to the needy rural people is a divine service. The Department is blessed with an opportunity to put all its efforts for prevention and control of Water Borne Diseases in rural habitations by providing safe water.

I am glad to know that the Rural Water Supply & Sanitation Department is bringing out the Laboratory manual on "Analysis of some basic parameters in Drinking Water". This manual is encompassing all the basic concepts of drinking water parameters with necessary information and detailed analytical methods for regular testing of drinking water samples in the Water Testing laboratories in the department. This manual is a hands on guide, not only to Laboratory personnel , but also to the Engineers in the department, common man, local bodies and concerned stake holders in line departments.

I would like to convey my heartfelt appreciations to the Project Director, State Water & Sanitation Mission and State Level Water Testing Laboratory, RWS&S dept., for making to present this laboratory manual in a very simple manner and easily understood by all the concerned laboratory personnel and engineers in the department.

I am very glad that this manual is throwing some light on new technological developments and instruments which leads to IOT and automation of the water quality monitoring on real time basis.

(Dr. N. Bharat Gupta)

Sri R.V. KRISHNA REDDY, M.Tech.,
Chief Engineer- III &
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Vijayawada.

Foreword

In order to safeguard the health of the people, drinking water must meet the quality standards prescribed by National standards (BIS) or International (WHO) guidelines. The concentrations of the Physico- Chemical, Microbial contaminants shall be ascertained according to the Standard procedures. Information regarding these procedures need to be clearly explained in simple and precise manner with theoretical background to the Laboratory personnel to enhance their knowledge.

I hope that the Laboratory personnel working in 107 water testing laboratories in the department, will extend all the required support in monitoring quality of all the drinking water sources periodically and suggest suitable precautionary measures to the Engineers and Panchayat Raj Institutes, for prevention and control of water borne diseases in rural habitations.

I would like to appreciate the efforts made by State Level Water Testing Laboratory RWS&S Dept., for bringing the manual with all the details of test methods along with theoretical background.

Hopefully, the information and test methods along with theoretical background, contained in the manual will provide necessary knowledge to the Laboratory personnel engaged in Water Testing Laboratories in the department.

(R.V.Krishna Reddy)

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Preface

"Science is not an encyclopedic body of knowledge about the universe. Instead it represents a process for proposing and refining theoretical explanations about the world that are subject to further testing and refinement. Science can tell us the way things are, but science cannot tell us the way things should be. The overall task relates to measurements aimed at protecting public health from drinking water risk, which amounts to risk management."

The Water Quality Monitoring wing in the Rural Water Supply & Sanitation Department, has immense potential to monitor the physico- chemical and bacteriological quality of about 2,53,000 drinking water sources in the rural habitations in the state. The Water Quality Monitoring Wing is periodically monitoring all the drinking water sources, with an overall objective of prevention & Control of Water Borne diseases in rural populations. The wing is assisting the department, time to time, in getting various schemes under projects sponsored by NRDWP, NABARD, World Bank etc..

In view of importance of the Water Quality Monitoring in the department, an attempt was made to bring the laboratory manual with all the details of test methods along with theoretical background, to enhance the knowledge of the laboratory personnel as well as engineers in the department. This manual includes all the test methods for physico-chemical and microbiological parameters in drinking water, which can be adopted by all the water testing laboratories in the RWS&S dept.

This course material aims to serve as a guidance manual and ready reckoner to the laboratory personnel in the department, for analysis of drinking water parameters

On behalf of State Level Water Testing Laboratory, RWS&S Dept., I Profoundly thank Sri A. Sateesh, Chief Chemist (Rtd.) RWS&S Dept., Telangana State, for his valuble guidance in finalizing the manual right from concept stage. I thank Smt. B. Swapna Reddy, and Sri R. Srikanth Reddy, Water Quality Consultants (WQM), for their hard work in providing valuable inputs on the Microbiology topics, in the manual.

M.V.S.R.KRISHNA MURTHY, M.Sc., M.B.A.,
Course co-ordinator,

CONTENTS

1. WATER AND IT'S IMPORTANCE

1.1 WATER IS LIFE

Water, that magical substance from which all life springs forth, is essential to the very existence of every life form on earth. Chemically water is a compound of oxygen and hydrogen with highly distinctive physical and chemical properties. It has chemical formula: H_2O. The role of water in the living organism has not changed since life's first creation in salt water, billions of years ago. It is a unique natural resource among all sources available on earth. Without water, the earth would look like the moon. No life form can be sustained without water on the planet.Earth is known as the "Blue Planet" because 70 percent of the Earth's surface is covered with water. It is essential for all the important activities like food production, industries like energy, production and manufacturing. It plays an important role in economic development andingeneral well being of anycountry. United Nations stated that water is a social and cultural commodity, not merely an economic commodity.

All living things need water to survive. The amount of water in the human body ranges from 50-75%. The percentage of water varies according to the age of a person and gender. Body compositionalso varies according to fitness level, since fatty tissue contains less water than lean tissue. The average adult malebodyconstitutes about 60% waterwhile that of an average adult woman is about 55% because women naturally have more fatty tissue than men. Overweight men and women have less percent water, than their leaner counterparts.

The percentage of water in infants is much higher, typically around 75-78% andit drops to 65% as the child grows to one year of age.Most of the body's water is in the intracellular fluid (2/3 of the body's water). The other fraction is in the extracellular fluid (1/3 of the water).

The amount of water varies, depending on the organ. Much of the water is in blood plasma. The amount of water in the human heart and brain is 73%, the lungs are 83%, muscles and kidneys are 79%, the skin is 64%, and the bones are around 31%.

1.2 What Is the Function of Water in the Body?

Water serves multiple purposes:

- Water is the primary building block of cells.
- It acts as an insulator, regulating internal body temperature. This is partly because water has a high specific heat, plus the body uses perspiration and respiration to regulate temperature.
- Water is needed to metabolize proteins and carbohydrates used as food. It is the primary component of saliva, used to digest carbohydrates and aid in swallowing food.
- Water insulates the brain, spinal cord, organs, and fetus. It acts as a shock absorber.
- Water is used to flush waste and toxins from the body via urine.

1.3 Drinking water should be :

- *Free from disease causing (pathogenic) organisms.*
- *Clear and transparent.*
- *Not saline.*
- *Free from offensive taste and odour.*
- *Free from chemical contaminants, which may have adverse effect on human health.*
- *Free from chemicals, which may cause corrosion to pipes and apparatus or stains on clothes and utensils.*

2. WATER QUALITY MONITORING

Introduction

Water quality is generally used to express the Physical, Chemical and Biological characteristics of water. The variations arises due to various natural and human interferences. This in general related to particular usage i.e., Drinking, Irrigation or Industries.

Water quality monitoring as defined by International Standards Organization is the programmed process of sampling, measurement and subsequent recording or signaling or both of various water characteristics, often with the aim of assessing conformity to specified objectives.

Objectives for Water Quality Monitoring:

1. To build up overall picture of the aquatic environment thus enabling pollution cause and effect to be judged.
2. To provide long-term background data against which future changes can be assessed.
3. To detect trends.
4. To provide warnings of potentially deleterious changes for specific use.
5. To collect sufficient data to perform in- depth analysis of an observed or predicted phenomenon.

2.1 Quality Assurance (QA):

Quality assurance, is a set of operating principles, if strictly followed during sample collection and analysis, will produce data of known and defensible quality. That is, the accuracy of the analytical result can be stated with high level of confidence. Quality assurance includes Quality Control and Quality Assessment.

The Set of operating principles in a Quality Assurance Program are,

1. Staff organization and responsibilities,
2. Sample control and documentation procedures,
3. Standard Operating Procedure for each analytical method (SOP).
4. Training requirements of Analysts.
5. Preventive maintenance procedures of Instruments & equipment.
6. Calibration Procedures & Corrective actions.
7. Internal quality control activities, Performance audits.

8. Data assessment, Validation, and reporting the results in standard units.

2.2 Quality Control (QC):

Quality Control (QC) is an intuitive effort put by the Analysts in the laboratory, to produce credible results. The good quality control program consists the following six elements:

1. Certification of Operator competence.
2. Recovery of known additions,
3. Analysis of externally supplied standards.
4. Analysis of reagents blanks.
5. Calibration of instruments with standards.
6. Analysis of duplicates and

2.3 Quality Assessment:

Quality assessment is the process of using external and internal quality control measures to determine the quality of the data produced by the laboratory. The measures aretest the recovery, bias, precision, detection limit and adherence to standard operation procedure requirements. It includes,

1. Performance evaluation samples,
2. Laboratory inter comparison samples,
3. Performance audits (to detect any deviations from the standard operating procedure so that corrective action can be taken)
4. Internal quality control.
5. Maintenance of control charts.

2.4 Quality indicators:

The principal indicators of data quality are 1. Bias and 2. Precision, which when combined, express its accuracy.

Bias is a measure of systematic error. It has two components: one due to the method and the other to a laboratory's use of method. **Precision** is a measure of the closeness with which the multiple analyses of a given sample agree with each other.

Accuracy is a measure of the agreement of a value of a variable in a sample with a known or "true" value. Random and systematic errors, during sampling and analysis of samples reduce accuracy. Accuracy is measured with standard reference materials with known values, either directly or as a spiked matrix sample. It is usually expressed as percent recovery.

Random errors refer to random variation or precision of the data, whereas systematic errors refer to bias, or values that are consistently higher or lower than the true value. Historically, the term accuracy has been used interchangeably with bias, but for the purpose of this guideline, accuracy includes precision as well as bias.

Difference between Quality Assurance & Quality Control

Quality Assurance	Quality Control
Anticipates problems before they occur	Responds to observed problems
Uses all available information to generate improvements	Uses ongoing measurements to make decisions on the processes or products
Is not tied to a specific quality standard	Requires a pre-specified quality standard for comparability
Is applicable mostly at the planning stage.	Is applicable mostly at the processing stage.
Is all-encompassing in its activities	Is a set procedure that is a subset of quality assurance

2.5 Checking the Correctness of the Analyses:

The following procedures for checking correctness of analyses are applicable specifically to water samples for which relatively complete analyses are made.1 These include pH,conductivity, total dissolved solids (TDS), and major anionic and cationic constituents that are indications of general water quality.

The checks described, do not require additional laboratory analyses. Three of the checks require calculation of the total dissolved solids and conductivity from measured constituents.

The sum total concentrations (in milligrams per liter) of constituents to calculate the total dissolved solids are as follows:

Total dissolved solids = 0.6 (alkalinity) + Na^+ + K^+ + Ca^{2+} + Mg^{2+} +

Cl^- + $SO4^{2-}$ + SiO_2 + NO^{3-} + F^-

Anion-Cation Balance

The anion and cation sums, when expressed as milliequivalents per liter, must balance because all potable waters are electrically neutral. The test is based on the percentage difference defined as follows:

$$\% \text{ difference} = 100 \frac{\Sigma \text{ cations} - \Sigma \text{ anions}}{\Sigma \text{ cations} + \Sigma \text{ anions}}$$

and the typical criteria for acceptance are as follows:

Anion Sum *meq/L*	Acceptable Difference
0–3.0	±0.2 meq/L
3.0–10.0	± 2%
10.0–800	±5%

Measured TDS = Calculated TDS

The measured total dissolved solids concentration should be higher than the calculated one because a significant contributor may not be included in the calculation. If the measured value is less than the calculated one, the higher ionic sum value may be suspectedfor possible errors and the sample should be reanalyzed. If the measured solids concentration is 20% higher than the calculated one, the low ionic sum may be suspected and selected constituents be reanalyzed. The acceptable ratio is as follows:

$$1.0 < \frac{\text{measured TDS}}{\text{calculated TDS}} < 1.2$$

Measured EC = Calculated EC

If the calculated electrical conductivity (EC) is higher than the measured value, reanalyze the higher ionic sum. If the calculated EC is less than the measured one, reanalyze the lower ion sum. The acceptable ratio is as follows:

$$0.9 < \frac{\text{calculated EC}}{\text{measured EC}} < 1.1$$

Measured EC and Ion Sums

Both the anion and cationic sums should be 1/100 of the measured EC value. If either of the two sums does not meet this criterion, that sum is suspect; reanalyze the sample. The acceptable criteria are as follows:

100 × anion (or cation) sum in meq/L = (0.9–1.1) EC

Calculated TDS to EC Ratio

If the ratio of calculated TDS to conductivity falls below 0.55, the lower ion sum is suspect; reanalyze it. If the ratio is above 0.7, the higher ion sum is suspect; reanalyze it. If re-analysis causes no change in the lower ion sum, an unmeasured constituent,

such as ammonia or nitrite, Iron and Zinc may be present at a significant concentration. If poorly dissociated calcium, Silica, Phosphates, borates and sulfate ions are present, the TDS may be as high as 0.8 times the EC and does not reflect proportionately in E.C. The acceptable criterion is as follows:

Calculated TDS/conductivity = 0.55–0.7

Silica and bulky groups such phosphates, borates etc., will enhance TDs but does not get reflected proportionately in E.C.

Measured TDS to EC Ratio

The acceptable criteria for this ratio are from 0.55 to 0.7. If the ratio of TDS to EC is outside these limits, measured TDS or measured conductivity is suspect; reanalyze.

Note:

a. Relations with many individual constituents and TDS can be established. Typically the constant is high for chloride-rich waters and low for sulphate rich waters.
b. If EC is checked at time of sampling and again prior to analysis in the laboratory, the change in EC is a measure for the 'freshness' of the sample.

2.6 Some electrical conductivity values for ions commonly found in water:

Current is carried by both cations and anions, but to a different degree. The conductivity due to divalent cations is more than that of mono-valentcations. However, it is not true for anions. The major dissolved components of ground waters include the anions such as bicarbonate, chloride, nitrate and sulphate, and the cations such as sodium, calcium, magnesium and potassium. These constituents are typically present at concentrations in the range of a few mg/L to several hundred mg/L.

The conductivity factors for major ions present in water are listed below.

Conductivity Factors for ions commonly found in water:

Name of the ion	Conductivity Factor µS/cm per mg/L	Multiplication factor for conversion of mg./lit. tomeq./lit.
Cations:		
Ca^{2+}	2.60	0.04990
Mg^{2+}	3.82	0.08229
K^{+}	1.84	0.02558
Na^{+}	2.13	0.04350
Anions :		
$HCO3^{-}$	0.715	0.01639
$CO3^{--}$	2.82	0.03333

Cl^-	2.14	0.02821
$SO4^{-2}$	1.54	0.02082
$NO3^-$	1.15	0.01613

The conductivity of a water sample can be approximated using the following relationship EC = S (Ci X fi) in which

EC = electrical conductivity, µS/ cm

Ci = concentration of ionic species in solution, mg / L

fi = conductivity factor for ionic species in µS/cm

Example

Given the following analysis of a water sample, estimate the EC value in µS/cm.

Cations: Ca2+=85.0 mg/L, Mg2+=43.0 mg/L, K+= 2.9 mg/L,
Na+ = 92.0 mg/L

Anions: HCO3-=362.0mg/L,Cl- =131.0 mg/L, SO42-=89.0 mg/L,
NO3-=20.0 mg/L

Calculate the electrical conductivity of each ion using the data given.

Name of the Ion	Conc. mg/L	Factor µS/cm per mg/L	Conductivity µS/cm
Ca2+	85.0	2.60	221.0
Mg2+	43.0	3.82	164.3
K+	2.9	1.84	5.3
Na+	92.0	2.13	196.0
HCO3-	362.0	0.716	258.8
Cl-	131.0	2.14	280.3
SO4-2	89.0	1.54	137.1
NO3-	20.0	1.15	23.0
Total	**824.9** (Total dissolved solids)		**1285.8** (Total E.C of the ions in µS/cm)

Electrical Conductivity = 1285.8 µS/cm = 1285.8 X 0.1 =
128.58 mS/m.

For the water sample given in the example calculate TDS and the corresponding constant 'A".

TDS in the sample = 824.9 mg/L. EC value = 1285.8 µS/cm.

TDS = A x EC

824.9 = A x 1285.8

A = 0.64

2.7 EXPRESSION OF RESULTS

International System of Units (SI) and chemical results are expressed in milligrams per liter (mg/L). Record only the significant figures. If concentrations generally are less than 1 mg/L, it may be more convenient to express results in micrograms per liter (µg/L). Use µg/L when concentrations are less than 0.1 mg/L.

Express concentrations greater than 10000 mg/L in percent,where, 1% is equal to 10 000 mg/L when the specific gravity is 1.00. In solid samples and liquid wastes of high specific gravity, a correction needs to be made if the results are expressed in parts per million (ppm) or percent by weight:

$$\text{ppm by weight} = \frac{\text{mg/L}}{\text{sp gr}}$$

$$\% \text{ by weight} = \frac{\text{mg/L}}{10\,000 \times \text{sp gr}}$$

In such cases, specific gravity need to be mentioned with results. The unit equivalents per million (epm), or the identical and less ambiguous term milligram-equivalents per liter, or milliequivalents per liter (me/L), can be valuable for making water treatment calculations and checking correctness of analysis by anion-cation balance. The equivalent weight, in turn, is defined as the weight of the ion (sum of the atomic weights of the atoms making up the ion) divided by the number of charges normally associated with the particular ion. The factors for converting results from milligrams per liter to milli equivalents per liter were computed by dividing the ion charge by weight of the ion. Conversely, factors for converting results from milli equivalents per liter to milligrams per liter were calculated by dividing the weight of the ion by the ion charge.

3. COLLECTION AND PRESERVATION OF WATER SAMPLES

Introduction:

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

General precautions:

1. Meet the requirements of the sampling program.
2. Handle sample so that it does not deteriorate or become contaminated or compromised before itis analyzed.
3. Ensure sampling equipment are clean and quality assured before use.
4. Use sample containers that are clean and free of contaminants.
5. Fill sample containers with/or without pre-rinsing(depending on the parameters to be analyzed)with sample, depending on the parameters to be analyzed.
6. Composite samples can be obtained by collecting over a period of time, depth, or at many different sampling points.
7. Make a record of every sample collected and identify every bottle.
8. Label, bottles and documentwith sufficient information for sample identification.
9. Before collecting samples from distribution systems, flush lines with 3-5 pipe volumes (or until water is being drawn from the main source).

1. Types of Samples:

a. Grab Sample:

A sample collected at a particular time and place can represent only the composition of the source at that time and place. Grab samples are single samples collected at a specific spot at a site over a short period of time (typically seconds or minutes). Thus, they represent a "snapshot" in bothspace and time of a sampling area. Discrete grab samples are taken at a selected location, depth, and time. Depth-integrated grab samples are collected over a predetermined part or the entire depth of a water column, at a selected location and time in a given body of water.

A sample can represent only the composition of its source at the time and place of collection. However, when a source is known to be relatively constant in composition over an extended time or over substantial distances in all directions, then the sample may represent a longer time period and/or a larger volume than the

specific time and place at which it was collected. In such circumstances, a source may be represented adequately by single grabsamples. Examples are protected groundwater supplies, water supplies receiving conventional treatment, some well-mixed surface waters, but very rarely, wastewater streams, rivers, large lakes, shorelines, estuaries, and groundwater come under this category.

b. Composite Sample:

Composite samples should provide a more representative sampling of heterogeneous matrices, in which the concentration of the analytes of interest may vary over short periods of time and/or space. Composite sample refers to a combination of grab samples collected at the same sampling point at different times. Composite samples can be obtained by combining portions ofmultiple grab samples or by using specially designed automatic sampling devices. Sequential (time) composite samples are collected by using continuous, constant sample pumping or by mixing equal water volumes collected at regular time intervals..

Advantages of composite samples:

- ❖ Reduces costs of analyzing a large number of samples.
- ❖ It is a representative of more samples of heterogeneous matrices and larger sample sizes when amounts of test samples are limited.

Disadvantages

- ❖ The analyte relationships in individual samples may be lost.
- ❖ The analytes may be diluted Potentially below detection levels
- ❖ There is a possibility of increased potential analytical interferences and increased possibility of analytes interactions.

c. Integrated *(discharge-weighted)* Sample:

For certain purposes, the information needed is provided best by analyzing mixtures of grab samples collected from different points simultaneously, or as nearly as possible, using discharge-weighted methods, such as, equal-width increment (EWI) or equal discharge-increment (EDI) procedures and equipment. An example of the need for integrated sampling occurs in a river or stream that varies in composition across its width and depth. To evaluate average composition or total loading, use a mixture of samples representing various points in the cross-section, in proportion to their relative flows. It is aMixture of grab samples collected from different points simultaneously, or as nearly so as possible. An example of need for integrated

sampling occurs in a river or stream that varies in composition across its width and depth.

2. ***Sample labels (including bar-code labels):*** Labels shall be used to prevent sample misidentification. Gummed paper labels or tags generally are adequate. The following minimum information shall be included : a unique sample number, sample type, name of collector, date and time of collection, place of collection, and sample preservative used (if any).
3. ***Sample seals:***Sample seals shall be used to detect unauthorized tampering of the samples, till the time of analysis.
4. ***Chain-of custody record:*** Chain-of-custody record shall be accompanied with each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time, and address of collection; sample type; requirements of sample preservation (if any); signatures of persons involved in the chain of possession; and inclusive dates and times of possession.
5. ***Sample analysis request sheet:***The sample analysis request sheet shall be accompanied with samples to the laboratory. The collector shall complete the field portion of such a form that includes most of the pertinent information noted in the log book. The laboratory portion of such a form is to be completed by laboratory personnel and includes: name of person receiving the sample,laboratory sample number, date of sample receipt, condition of each sample (i.e., if it is cold or warm, whether the container is full or not, color, if more than one phase is present, etc.) and determinations to be performed.
6. ***Sample delivery to the laboratory:*** *The* sample(s) shall be delivered to the sample custodian in the laboratory as soon as practicable after collection. Ensure that samples are accompanied by a complete chain-of-custody record and a sample analysis request sheet.
7. ***Receipt and logging of sample:***In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain-of-custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and/or computerized laboratoryinformation management system, and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is assigned to an analyst.

8. ***Assignment of sample for analysis:***The laboratory supervisor usually assigns the sample for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.
9. ***Disposal:***Hold samples for the prescribed amount of time for the project or until the data have been reviewed and accepted. Document the disposition of samples. Ensure that disposal is in accordance with local, state, and U.S. EPA approved methods.
10. **Sample Container:**

Water samples should be collected in a glass or plastic bottles that have been cleansed and rinsed carefully, given a final rinse with de-ionized or distilled water, and sterilized. Glass containers are to be used for all organic analyses such as volatile organics ,semi volatile organics, pesticides , PCB's and oil and grease. Some analytes are light sensitive, collect them in amber glass containers to minimize photo degradation.

Sample containers:

11. **Sample Preservation:**

a. Certain cations are subject to loss by adsorption on, or ion exchange with the walls of the glass containers, these includes: Al,Cd,Cr,Cu,Fe,Pb,Mn,Ag,and Zn. Those are best collected in a separate clean bottle and acidified with nitric acid to pH below 2.0 to minimize precipitation and adsorption on container walls.

b. Changes in the pH-alkalinity-carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values of calcium and total hardness. Hardness may be preserved by adding nitric acid to pH <2 and also by collecting the sample without any air bubble inside the sample container.
c. Changes caused by growth of microorganisms are greatly retarded by keeping the sample at a low temperature (<4° C), but above freezing.
d. While sampling chlorinated water 0.5 ml of sodium Thiosulphate solution (18 gm/L) should be added to sampling bottles to neutralize the residual chlorine present in water.

12. Time interval between collection and Micro biological examination :

1. Microbiological examination of water samples should be started as soon as possible after collection.
2. Samples for microbiological examination should be kept preferably at <10° C, away from the direct sunlight, during transport, if they can't be analyzed within 6 hrs. after collection.
3. Analyze samples on day of receipt whenever possible and refrigerate overnight if arrival is too late for analyzing on same day.

13. Sampling Methods for Micro biological examination :

A. Sampling from a tap or pump outlet :

a) Remove any attachments from tap that may cause splashing.

b) Wipe off the dirt from outside the tap.

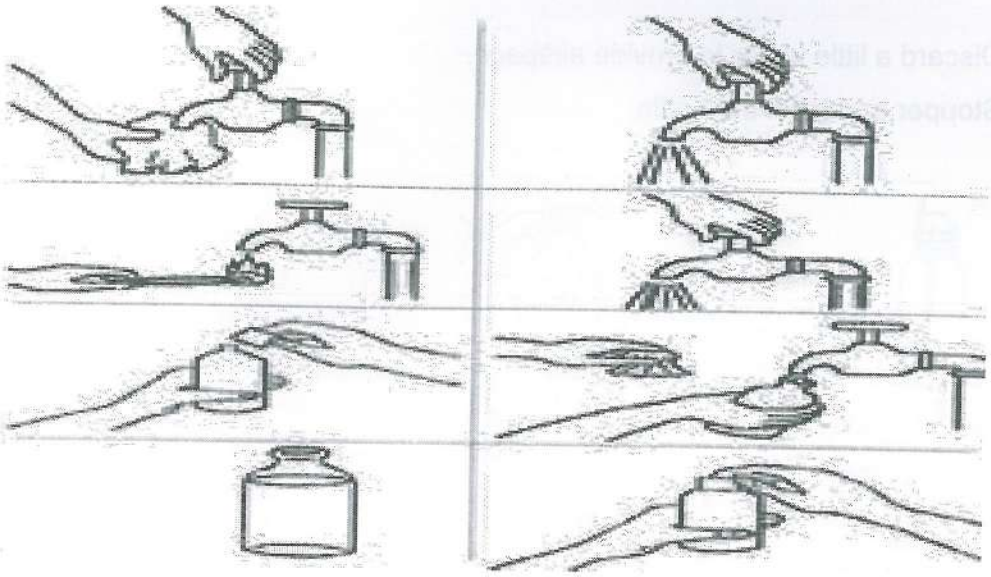
c) Turn on the tap at maximum flow rate and let the water flow for 1-2 minutes.

d) Sterilize it for a minute with flame using gas burner, lighter or ignited cotton wool soaked in spirit.

e) Open the tap and allow water to flow at medium rate for 1-2 minutes.

f) Open the container for collecting the sample and fill the water by holding the bottle under the water jet. Leave a small airspace to facilitate shaking at the time of inoculation prior to analysis.

g) Stopper the cap and label the container.

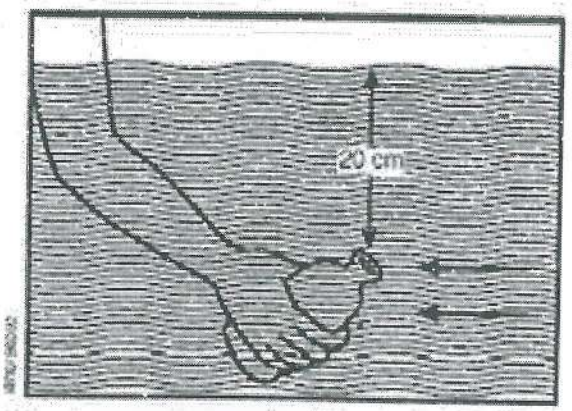


B. Sampling from reservoir, lake or pond:

a. Open the bottle under sterilized conditions.

b. Fill it by holding it by the lower part, submerging it to a depth of about 20 cms, with the mouth facing slightly upwards. If there is a current, the bottle should face the current.

c. Stopper the bottle and label it.



C. Sampling from a dug well:

a. Attach a stone of suitable size to the sampling bottle with a piece of string.

b. Tie a 20 meter length of clean string on the bottle and to a stick.

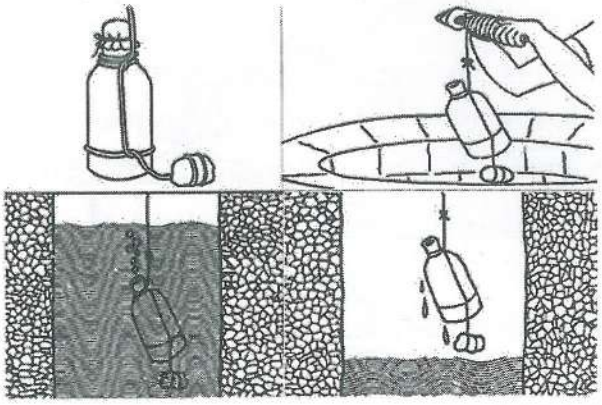
c. Open the bottle as described above and lower into the well.

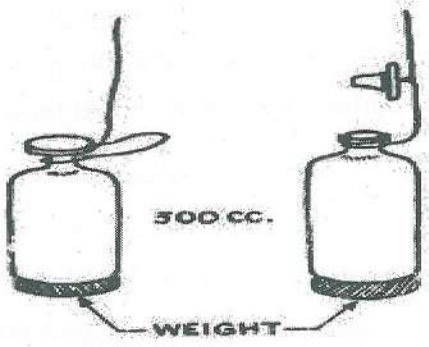
d. Immerse the bottle completely in water without touching the sides of the well and lower it down to the bottom of the well.

e. Pull it out when the bottle is filled.

f. Discard a little water to provide airspace.

g. Stopper and label the bottle.





PHYSICO – CHEMICAL PARAMETERS

1. COLOUR

Colour in Water may result from the presence of natural metallic ions (Iron and Manganese), humus and peat materials, plankton, weeds and industrial wastes. The Term **"Colour"** is used here to mean **true colour**, that is the colour of the water , from which turbidity has been removed. The term "**apparent colour**" includes not only colour due to substances in solution, but also due to suspended matter. Apparent colour is determined on the original sample without filtration or centrifugation.

One major factor that affects the colour of natural surface water is pH. The increase in colour with increasing pH of the test sample is commonly referred to as the "indicator effect," and it is widely recommended that the pH of the sample be recorded together with the colour measurement to allow for this effect.

Samples Pretreatment:

Samples require pretreatment to remove suspended matter and turbidity in order to determine its true color. Filtration method yields reproducible results. However some filtration methods also remove true colour. Centrifugation avoids interaction of color with filter materials; but results vary with the sample nature, size and speed of the centrifuge.

Test Method:

Visual comparison method:

Colour is determined by visual comparison of the sample with known concentrations of colored solutions. The Platinum- Cobalt method of measuring color is the standard method. This method is applicable to nearly all samples of potable water.

One true colour unit (TCU), or platinum-cobalt unit, corresponds to the amount of colour exhibited under the specified test conditions by a standard solution containing 1.0 mg of platinum per litre in the form of Chloroplatinate ion. Thus, a sample exhibiting a colour of 15 TCU has a closer colour match with a standard containing 15 mg of platinum per litre than with standards containing 14 or 16 mg/L. A colour of 15 TCU can be detected in a glass of water by most consumers, and 5 TCU will be apparent in large volumes of water, such as in a bathtub; few people can detect a colour level of 3 TCU. Waters containing natural color are yellow-brownish in appearance. Solutions of potassium chloroplatinate (K_2PtCl_6) tinted with small amounts of cobalt chloride yield colors that are very much like the natural colors.

Apparatus:

1. Nessler tubes: matched, 50 – ml, tall form.
2. pH meter for determining the pH of the sample.

Preparation of standard solutions:

Dissolve 1.246 gms. Of Potassium Chloroplatinate, K_2PtCl_6 (equivalent to 500mg. of metallic Pt) and 1.00 gms of crystallized Cobaltous Chloride, $CoCl_2.6H_2O$ (equivalent to about 250 mg. of metallic Co) in distilled water with 100 ml. Con.HCl and dilute to 1000 ml.of distilled water. This stock standard has a color of 500 units.

Prepare standards having colors of 5,10,15,20,25,30,35,40,45,50,60 and 70 by diluting 05,1.0,2.0,2.5,3.0,3.5,4.0,4.5,5.0,6.0,7.0 ml. of stock color standard with distilled water to 50 ml in Nessler tubes.

Procedure: Observe the sample color by filling a matched nessler tube to 50 ml. mark with sample and comparing it with standards. Look vertically downwards through tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid. If the color exceeds by70 units, dilute sample with distilled water in known proportions, until the color is within the range of the standards.

Calculation:

Color units = AX50/B

Where:

A = Estimated color of diluted sample and

B = ml. sample taken for dilution.

Guidelines of Colourin drinking water:

As per IS 10500 :2012, in Drinking water, the acceptable limit is 5 Pt-CO unit and permissible limit in the absence of alternate source is 15 Pt-CO units.

2. ODOUR

Odour depends on contact of a stimulating substance with the appropriate human receptor cell. Water is neutral medium, always present on or at the receptors that perceive sensory response. In its pure form, water can produce any odour or taste sensations. Human beings and animals avoid many potentially toxic foods and waters because of adverse sensory response. These senses often provide the first warning potential hazards in the environment.

Odour is recognized as a quality factor affecting acceptability of drinking water. Most organic and some inorganic chemicals contribute taste or odour. These chemicals may originate, from municipal and industrial waste discharges, from natural sources such as decomposition of vegetable matter or from associated microbial activity and from disinfectants or their products. The ultimate odour testing device is the human nose.

Odour tests are performed to provide qualitative descriptions and approximate quantitative measurements of odour intensity. The method for measurement of odour intensity is the *"Threshold odour Test"*.

Test Method:

Threshold Odour Test is done by diluting a sample with odour – free water until the least definitely perceptible odour is achieved. There is no absolute threshold odour concentration, because of inherent variation in individual olfactory capability. Water Samples should be collected in glass bottles with glass or TFE – lined closures. The test should be completed as soon as possible after the sample collection. Threshold odour values vary with temperatures. For most waters sample temperature of 60°C is permitted for detection of odours - 60°C is the standard temperature for hot threshold odour tests. For some samples - because the odour is too fleeting or there is excessive heat sensation, a standard test temperature of 40°C is permitted. The Threshold OdourNumber (TON) is the greatest dilutions of the sample with odour free water yielding a definitely perceptible odour. The TON is the dilution ratio at which the odour is just detectable. The total volume of the sample and odour- free water in each test should be 200 ml.

Procedure:

1. Determine approximate range of the threshold odour number by adding 200 mL, 50 mL, 12 mL, and 2.8 mL sample to separate 500-mL glass-stoppered Erlenmeyer flasks

and odour-free water to make a total volume of 200 mL. Use a separate flask containing only odour-free water as reference for comparison. Heat dilutions and reference to a desired test temperature (40°C or 60°C).

2) Shake flask containing odour-free water, remove stopper, and sniff vapors. Test sample containing least amount of odour-bearing water in the same way. If odour cannot be detected in first dilution, repeat above procedure using sample containing next higher concentration odour-bearing water, and continue this process until odour is detected clearly.

The Dilutions and corresponding TON are presented in the table.

Sample Volume Diluted to 200 mL	Threshold Odor Number (TON)	Sample Volume Diluted to 200 mL	Threshold Odor Number (TON)
200	1	8.3	24
100	2	5.7	35
70	3	4	50
50	4	2.8	70
35	6	2	100
25	8	1.4	140
17	12	1	200

Threshold Odour Numbers TON = (A + B)/ A

A - Volume of Sample with odour

B - Volume of Pure Water with no odour Added

If A was a 100 ml sample and 100 ml of odour- free water had to be added, at which the odour is just detectable. TON = (100 + 100)/ 100 = 2.

If the sample being tested required more dilution than is provided in the table, the intermediate dilution consisting of 20 ml. of sample , diluted with 200 ml. of Odour – free water is to be prepared. This dilution is to be used for Threshold odour determination. The TON obtained is tobe multiplied with 10 to correct the intermediate dilution.

3. Turbidity

Turbidity is a principal physical characteristic of water and is an expression of the optical property that causes light to be scattered and absorbed by particles and molecules rather than transmitted in straight lines through a water sample. It is caused by suspended matter or impurities that interfere with the clarity of the water. These impurities may include clay, silt, finely divided inorganic and organic matter, soluble colored organic compounds, and plankton and other microscopic organisms. Typical sources of turbidity in drinking water include the following:

- Waste discharges;
- Runoff from watersheds, especially those that are disturbed or eroding;
- Algae or aquatic weeds and products of their breakdown in water reservoirs, rivers, or lakes;
- Humic acids and other organic compounds resulting from decay of plants, leaves, etc. in water sources; and
- High iron concentrations which give waters a rust-red coloration (mainly in ground water and ground water under the direct influence of surface water).
- Air bubbles and particles from the treatment process (e.g., hydroxides, lime softening)

Simply stated, turbidity is the measure of relative clarity of a liquid. Clarity is important when producing drinking water for human consumption and in many manufacturing uses.

Once considered as a mostly aesthetic characteristic of drinking water, significant evidence exists that controlling turbidity is a competent safeguard against pathogens in drinking water.

Measurement :

The method is based on a comparison of the intensity of light scattered by the sample, under the defined conditions, with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of the scattered light, the higher the turbidity.The Turbidity measurement was standardardised in the 1970's, when the Nephelometric turbidity meter, or Nephelometer, was developed which determines turbidity by the light scattered at an angle of 90° from the incident beam. A 90° detection angle is considered to be the least sensitive to variations in particle size. Nephelometry has been adopted by Standard Methods as the preferred

means for measuring turbidity because of the method's sensitivity, precision, and applicability over a wide range of particle size and concentration. The nephelometric method is calibrated using suspensions of formazin polymer, as a primary standard. The value of 40 nephelometric units (NTU) is approximately equal to 40 JTU. The preferred expression of turbidity is NTU.

Preparation of Stock Primary standard Formazine Suspension:

Solution – I : Dissolve 1.00 gms of Hydrazine Sulfate, (NH2)2. H2SO4 in a distilled water and dilute to 100 ml. in a volumetric flask.

Solution – II : Dissolve 10.0 gms. Of Hexamethylenetetraamine(CH2)6N4, in a distilled water and dilute to 100 ml. in a volumetric flask.

In another volumetric flask mix 5.0 ml solution I and 5.0ml Solution II and let the mixture stand for 24 Hours at 25± 3°C. This results in 4000-NTU suspension. This Primary standard stock suspension should be transferred in an amber glass bottle for storage, which is stable up to 1 year.

Make dilutions from this stock solutions.

Turbidity can be measured using either an electronic turbidity meter or a turbidity tube. Turbidity is usually measured in nephelometric turbidity units (NTU) or Jackson turbidity units (JTU), depending on the method used for measurement. The two units are roughly equal.

There are many different types of electronic turbidity meter available.It is impossible to give general guidelines on their use. Manufacturers' instructions are to be referred for use and maintenance of these meters.

Effects of Turbidity:

- Turbidity diffuses sunlight and slows photosynthesis. Plants begin to die, reducing the amount of dissolved oxygen and increasing the acidity (decaying organic material produces carbonic acid, which lowers the pH level). Both of these effects harm aquatic animals.
- Turbidity raises water temperature because the suspended particles absorb the sun's heat. Warmer water holds less oxygen, thus increasing, the effects of reduced photosynthesis. In addition, some aquatic animals may not adjust well to the warmer water, particularly during the egg and larval stages.

- Highly turbid water can clog the gills of fish, stunt their growth, and decrease their resistance to diseases.
- The organic materials that may cause turbidity can also serve as breeding grounds for pathogenic bacteria. When drinking water reservoirs are turbid, the water treatment plant usually filters the water before disinfecting it.

Turbidity's Significance to Human Health:

Excessive turbidity, or cloudiness, in drinking water is aesthetically unappealing, and may also represent a health concern. Turbidity can provide food and shelter for pathogens. If not removed, turbidity can promote re-growth of pathogens in the distribution system, leading to waterborne disease outbreaks, which have caused significant cases of gastroenteritis. Although turbidity is not a direct indicator of health risk, numerous studies show a strong relationship between removal of turbidity and removal of protozoa.

The particles of turbidity provide "shelter" for microbes by reducing their exposure to attack by disinfectants. Traditional water treatment processes have the ability to effectively remove turbidity when operated properly.

Guidelines of Turbidity in drinking water:

- As per IS 10500 :2012, in Drinking water, the acceptable limit is 1NTU and permissible limit in the absence of alternate source is 5NTU. Turbidity of more than 5 NTU/JTU would be noticed by users and may cause rejection of the supply.
- Where water is chlorinated, turbidity should be less than 5 NTU/JTU and preferably less than 1 NTU/JTU for chlorination to be effective.

4. p^H Value

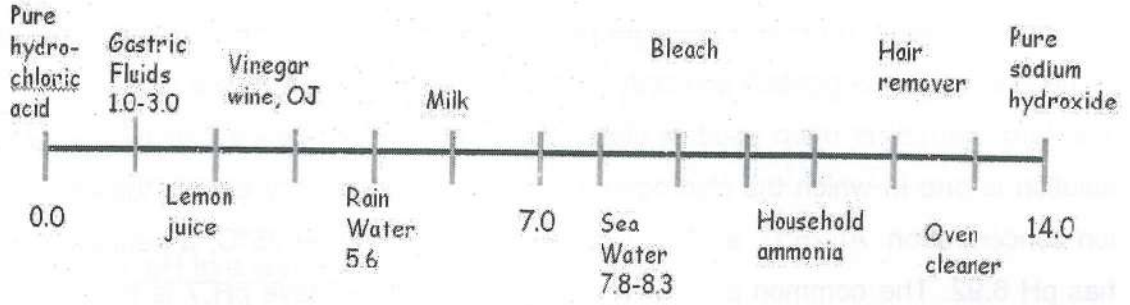
What is pH?

pH is a measurement of how acidic or how basic (alkaline) a solution is. When substances dissolve in water they produce charged molecules called ions. Acidic water contains extra hydrogen ions ($H^{+)}$ and basic water contains extra hydroxyl (OH^-) ions. Thus at a given temperature, the pH indicates the intensity of the acidic or basic character of the solution, in terms of Hydrogen ion concentration.

pH is measured on a scale of 0 to 14. Water that is neutral has a pH of 7. Acidic water has pH values less than 7, with 0 being the most acidic. Likewise, basic water has values greater than 7, with 14 being the most basic.

The pH of natural water usually lies in the range of 4-9. Majority of natural waters are slightly basic in nature, due to presence of bicarbonates and carbonates of alkali and alkaline earth metals. pH value in natural waters is governed by the Carbon dioxide and Bicarbonate/ Carbonate equilibrium.

You might expect rainwater to be neutral, but it is actually somewhat acidic. As rain drops fall through the atmosphere, they dissolve gaseous carbon dioxide, creating a weak acid. Pure rainfall has a pH of about 5.6.



pH is a measure of the relative amount of hydrogen and hydroxide ions in an aqueous solution. In any collection of water molecules a very small number will have dissociated to form hydrogen (H^+) and hydroxide (OH^-) ions:

$$H2O \leftrightarrow H^+ + OH^-$$

The number of ions formed is small. At 25°C fewer than 2×10^{-7} % of the water molecules have dissociated. In terms of molar concentrations, water at 25°C contains $1x10^{-7}$ moles per liter of hydrogen ions and the same concentration of hydroxide ions.

In any aqueous solution, the concentration of hydrogen ions multiplied by the concentration of hydroxide ions is constant. Stated in equation form:

$$K_w = [H^+] [OH^-] \qquad (1)$$

where the brackets signify molar concentrations and K_w is the dissociation constant for water. The value of Kw depends on temperature. For example, at 25°C $K_w = 1.00 \times 10^{-14}$ and at 35°C $K_w = 1.47 \times 10^{-14}$.

Acids and bases, when dissolved in water, simply alter the relative amounts of H^+ and OH^- ions in solution. Acids increase the hydrogen ion concentration and decrease the hydroxide ion concentration, because the product [H+] [OH-] must remain constant. Bases have the opposite effect. They increase hydroxide ion concentration and decrease hydrogen ion concentration. For example, suppose when an acid is added to water at 25°C, the acid raises the H+ concentration to 1.0×10^{-4} moles/liter. Because $[H^+] [OH^-]$ must always equal 1.00×10^{-14},the $[OH^-]$ will be 1.0×10^{-10} moles/liter.

pH is another way of expressing the hydrogen ion concentration. pH is defined as follows:

$$pH = -\log [H+] \qquad (2).$$

Therefore, if the hydrogen ion concentration is 1.0×10^{-4} moles/liter, the pH is 4.00.

Finally, it should be noted that equation (2) is somewhat misleading. The equation implies that pH is a measure of concentration. In fact, pH is really a measure of ion activity. Concentration and activity are not the same, but they are related.

The term neutral is often used in discussions about acids, bases and p^H. A neutral solution is one in which the Hydrogen ion concentration exactly equals the Hydroxide ion concentration. At 25°C, a neutral solution has pH 7.00. At 35°C, a neutral solution has pH 6.92. The common assertion that neutral solutions have pH 7 is not true. The statement is true only if the temperature is 25°C.

The ionic product of water is,$[H^+] [OH^-] = K = 1X10^{-14}$ at 25° C

At equilibrium,$[H^+] = [OH^-] = 1.005 X10^{-7}$.

Definition of the pH value :

According to Sörenson the pH is defined as the negative (base 10) logarithm of the H+ Ion concentration, i.e.:$pH = - \log_{10} [H^+]$

If the H^+ ion concentration changes by the factor of ten, the pH value changes by one unit.

This definition of the pH value is sufficiently accurate only for dilute solutions, since only then are the concentration and activity of the solution equal. In many cases, however, the activity coefficient is smaller than one (the activity coefficient of the hydrogen ion is not measurable in real solutions). It is common to refer to H^+ ions in connection with pH values, although the correct term is the Hydronium (oxonium) ion ($H3O^+$):

$$H^+ + H2O \leftrightarrow H_3O^+$$

The hydrogen ion normally exists in its associated form:

As pH is expressed in logarithmic scale, a drop of 1 unit of pH value is equivalent to an increase of 10 times acid intensity. So that water with p^H of 6 is 10 times more acidic than water with a p^H of 7, and water with a pH of 5 is 100 times more acidic than water with a p^H of 7.

p^H does not measure the total acidity or alkalinity of the solution. This is explained by comparing the pH of 0.1 solutions of HCl and Acetic acid, which give the same neutralizing value. The p^H of 0.1N HCl is 1.1, due to high degree of Ionization and p^H of 0.1 N Acetic acid is about 2.9, due to low degree of ionization. The p^H of 0.1 N Solutions of few standard acids and bases at 25°C are given below.

Acids	p^H	Bases	p^H
HCl (0.1N)	1.1	NaOH (0.1N)	13.0
H_2SO_4 (0.1N)	1.2	Ammonia (0.1N)	11.1
Acetic Acid (0.1N)	2.9	Na_2CO_3 (0.1N)	8.4
Boric acid (0.1N)	5.2		

Measuring pH in a water sample:

There are many types of pH meters are available in the market. It is almost impossible to give general instructions on their usage. Manufacturer's instructions are to be referred for use and maintenance of these meters.

Procedure:

The pH meter should be standardized each time it is used with a buffer of known pH, preferably one closest to the desired final pH. To calibrate the pH meter, rinse the electrode with deionized water, and place the electrode in a standard solution, e.g., pH 7. Turn the selector to "pH". Adjust the pH meter to the appropriate pH. Rinse electrode

with deionized water and place in a second standard buffer solution. The choice of the second standard depends on the pH value to be measured. The standard pH buffers used should be 7 and 10, if the final pH desired, for example, is 8.5. the standard pH buffers used should be 4 and 7, If the pH desired is 5.5,. After the measuring the pH values of the samples ,return the electrode to the soaking solution.

Precautions to be taken:

- When rinsing the electrode, never wipe the end, but blot gently since wiping can create a static electric charge, which can cause erroneous readings,
- Make sure the solution you are measuring is at room temperature since the pH can change with a change in temperature.
- The pH-sensitive glass membrane is very thin and can be easily broken. Do not drop the electrode or bump it on the bottom of the beaker when immersing it in a solution.
- The glass membrane must be thoroughly hydrated to work properly. Do not allow the electrode to remain out of water any longer than necessary. When the electrode is not in use, keep it immersed in the pH 7.00 buffer. Do not put the electrode down on the desk.

Effects of pH on animals and plants:

- Most aquatic animals and plants have adapted to life in water with a specific pH and may suffer from even a slight change.
- Even moderately acidic waters (low pH) may reduce the hatching success of fish eggs, irritate fish and aquatic insect gills and damage membranes.
- Water with extremely high or low pH is deadly. A pH below 4 or above 10 will kill most fish and very few animals can tolerate waters with a pH below 3 or above 11.
- Amphibians are particularly vulnerable to low pH, probably because their skin is so sensitive to pollutants.

5. Electrical conductivity

1 Introduction

As in the case of metallic conductors, electrical current can flow through a solution of electrolyte also. In metallic conductors, electric current is carried out by electrons, while in solution of electrolyte, electric current is carried out by ions.

Pure water is a poor conductor of Electricity. Acids, bases and salts in water make it relatively good conductor of electricity and such substances are called electrolytes. The electrolytes in solution dissociates with Cations and Anions and imparts Electrical conductivity. Thus higher the concentration of the Electrolytes, the more is its electrical conductivity. This gives rapid method to get an idea about dissolved solids in water.

The Major ions , which impart conductivity in water are:

Anions: Cl^-, SO_4^{--}, CO_3^{--}, HCO_3^-, NO_3^-.

Cations: Ca^{2+}, Mg^{2+}, Na^+, K^+.

Electrical conductivity (EC) is a numerical expression of the ability of water to conduct an electric current and depends on:

- Concentration/Number of the ions (higher concentration, higher EC)
- Temperature of the solution (high temperature, higher EC)
- Specific nature of the ions (higher specific ability and higher valency, higher EC)

It is related to the concentration of ionized substances in the water. Most dissolved inorganic substances in water are in the ionized form and hence contribute to conductance.

Conductivity changes with storage time and temperature. The measurement should therefore be made in situ (dipping the electrode in the stream or well water) or in the field directly after sampling. The determination of the electrical conductivity is a rapid and convenient means of estimating the concentration of ions in solution. Since each ion has its own specific ability to conduct current, EC is only an estimate of the total ion concentration.

Equations and dimensions

Ohm's law defines the relation between potential (V) and current (I). The resistance (R) is the ratio between V and I:

$$R=V/I \qquad (1)$$

The resistance depends upon the dimensions of the conductor, length, L, in cm, cross sectional area, A, in $cm^2$and the specific resistance,(S) in ohm.cm, of the conductor:

$$R= S X L/A \quad (2)$$

In the present case our interest is in specific conductance or electrical conductivity "k" (which is the preferred term), the reciprocal of specific resistance, in 1/ohm.cm or Siemens per centimeter, S/cm, which can be thought of as the conductance offered by 1 $cm^3$of electrolyte.The term specific conductance (SC) has sometimes been used to refer to electrical conductivity measured at a specified reference temperature. However, SC is considered synonymous with electrical conductivity by the International Union of Pure andApplied Chemistry:

Conductance (C) = 1/R = 1/S X [1/(L/A)]

$$C= k X [1/(L/A)] \quad (3)$$

$$And\ k = C XL/A$$

Specific conductivity (k) is simply the product of measured conductivity (C) and the electrode cell constant (L/A), where L is the length of the column of liquid between the electrode and A is the area of the electrodes (see Figure 1).

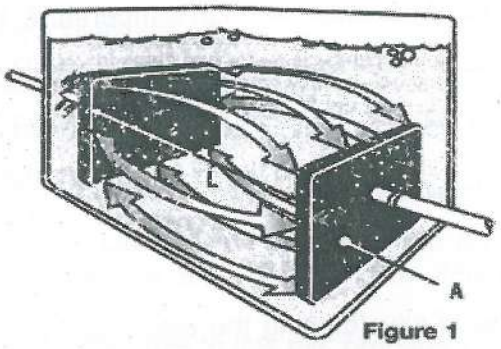


Figure 1

The resistance of the electrolyte is measured across two Chemically inert plates dipped in the liquid and held at a fixed distance apart in a conductivity cell. The ratio L/A for the cell is called cell constant, K_c, and has the dimensions 1/cm. The value of the constant is determined by measuring the resistance of a standard solution of known conductivity:

$$K_c = R X k \quad (4)$$

Unit of measurement and reporting:

In the international system of units (SI) the electrical conductivity is expressed in Siemens which is the reciprocal of resistance in ohm. The older unit for conductance was mho. Report conductivity as milli Siemens per meter at 25°C (mS.m-1).

The Apparatus:

An apparatus called a conductivity meter that consists of a conductivity cell and a meter, used to measure conductivity. The conductivity cell consists of two electrodes (platinum coatedplates) rigidly held at a constant distance from each other and are connected by cables to the meter. The source of electric current in the meter applies a potential to the plates and the meter measures the electrical resistance of the solution. In order to avoid change of apparent resistance with time due to chemical reactions (polarisation effect at the electrodes) alternating current is used. Some meters read resistance (ohm) while others read in units of conductivity (milli-Siemens per meter). Platinised electrodes must be in good condition (clean, black-coated). The cell should always be kept in distilled water when not in use, and thoroughly rinsed in distilled water after measurement.

The cell constant (calibration)

The design of the plates in the conductivity cell (size, shape, position and condition) determines the conductivity measured and is reflected in the so-called cell constant (Kc), Typical values for Kc are 0.1 to 2.0. The cell constant can be determined by using the conductivity meter to measure the resistance of a standard solution of 0.0100mol/L potassium chloride (KCl). The conductivity of the solution (141.2 mS/m at 25°C) multiplied by the measured resistance gives the value of Kc, Equation 4. The cell constant is subject to slow changes in time, even under ideal conditions. Thus, determination of the cell constant must be done regularly.

Most of the modern conductivity meters have a facility to calculate the specific conductivity at 25°C using a built in temperature compensation from 0 to 60°C. The compensation can be manual (measure temperature separately and adjust meter to this) or automatic (there is a temperature electrode connected to the meter).

Reagents & standards:

Conductivity water :

Conductivity of the water used for preparation of standard KCl solution should be less than 1 µs/cm.

Standard Potassium Chloride Solution (0.01M):

Dissolve 745.6 mg. of anhydrous KCl(AR grade) powder in conductivity water and make up to 1 litre at 25°C. This solution has specific conductance of 1413 µs/cm at 25 °C and shall be used as reference standard solution.

Measurement Procedure:

The procedure for calibration of the Conductivity meter and then measurement of conductivity of water samples, shall be followed as per the instruction manual supplied with instrument and then results may be expressed in ms/m or µs/cm.

6. TOTAL DISSOLVED SOLIDS

Definition:

Water is a good solvent and picks up impurities easily. Pure water is often called the universal solvent. "Dissolved solids" refer to any minerals, salts, metals, cations or anions dissolved in water.

Total dissolved solids (TDS) is the term used to describe the inorganic salts and small amounts of organic matter present in water. The principal constituents are usually calcium, magnesium, sodium, and potassium cations and carbonate, hydrogen carbonate, chloride, sulfate, and nitrate anions.

The total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) in the water. Therefore, the total dissolved solids test provides a qualitative measure of the amount of dissolved ions, but does not tell us the nature or ionic relationships. In addition, the test does not provide us insight into the specific water quality issues, such as, elevated hardness, salty aste, or corrosiveness. Therefore, the total dissolved solids test is used as an indicator test to determine the general quality of the water. The sources of total dissolved solids can include all of the dissolved cations and anions, but the following table can be used as a generalization of the relationship of TDS to water quality problems.

Cations combined with Carbonates :$CaCO_3$, $MgCO_3$etc	Associated with hardness, scale formation, bitter taste
Cations combined with Chloride: NaCl, KCl	Salty or brackish taste, increase corrosivity

Organoleptic properties:

The presence of dissolved solids in water may affect its taste (1). The palatability of drinking water has been rated by panels of tasters in relation to its TDS level as follows:

Excellent - less than 300 mg/litre;

Good - Between 300 and 600 mg/litre;

Fair - Between 600 and 900 mg/litre;

Poor - Between 900 and 1200 mg/litre; and

Unacceptable - Greater than 1200 mg/litre .

Water with extremely low concentrations of TDS may also be unacceptable because of its flat and insipid taste.

Health Effects:

An elevated total dissolved solids (TDS) concentration is not a health hazard. The TDSconcentration more of an aesthetic rather than a health hazard and interfere with washing clothes and corroding plumbing fixtures. An elevated TDS indicates that the water is corrosive, salty or brackish taste, result in scale formation, and interfere and decrease efficiency of hot water heaters.

Measurement of TDS:

The two principal methods of measuring total dissolved solids are gravimetry and conductivity. Gravimetric methods are the most accurate and involve evaporating the liquid solvent and measuring the mass of residues left. This method is generally the best, although it is time-consuming. If inorganic salts comprise the great majority of TDS, gravimetric methods are appropriate.

The total dissolved solids concentration can be related to the conductivity of the water, but the relationship is not a constant. The relationship between total dissolved solids and conductivity is a function of the type and nature of the dissolved cations and anions. Ions from the dissolved solids in water create the ability for that water to conduct an electrical current, which can be measured using a conventional conductivity meter or TDS meter. When correlated with laboratory TDS measurements, conductivity provides an approximate value for the TDS concentration, usually to within ten-percent accuracy.

The relationship of TDS and specific conductance of groundwater can be approximated by the following equation:

$$TDS = k_e \times E.C$$

where TDS is expressed in mg/L and EC is the electrical conductivity in micro siemens per centimeter at 25° C. The correlation factor k_e varies between 0.55 and 0.7.

For example, a NaCl solution and KCl solution with a conductivity of 10000 µs/cm will have different total dissolved solids concentration.

Testing :

Total Dissolved Solids (TDS): In a laboratory setting, the total dissolved solids is determined by filtering a measured volume of sample through a standard glass fiber filter. The filtrate (i.e., filtered liquid) is then added to a pre weighed ceramic dish that is placed in a drying oven at a temperature of 103° C. After the sample dries, the temperature is increase to 180° C to remove an occluded water, i.e., water molecules trapped in mineral matrix.

Gravimetric method for measuring the Total Dissolved Solids:

Step 1.

Filter your water sample through a rinsed and dried glass fiber filter discs or other products which give demonstrably equivalent results). Collect the filtrate (liquid) and rinse water in a flask. The minimum sample volume should be 100 ml and you should use at least 3 rinses of 20 to 30 ml volumes. Record weight of container and volume of filtrate (do not include the volume of the rinse water). The rinse water should be de-ionized water.

Step- 2.

Transfer the filtrate to a ceramic or glass Pyrex container. The container should be weighed to the nearest 0.0000 g and place the container in the drying oven, which is set at 103 °C. Add the filtrate to the container and allow the sample to stay in the oven at 103° C for 24 hours. If possible, increase the temperature of the drying oven to 180° C and allow the sample to dry for up to 8 hours. Remove the container. After removing from the drying oven, the sample should be placed in a desiccators to cool in a dry air environment for at least 3 to 4 hours. If the sample cooled in a moist environment, the sample would increase in weight because of the addition of water vapor from the air.

Step- 3:

After the container cools, reweigh the container at least three times to the nearest 0.0000 g (Recording your data).Subtract the initial weight (in grams) of the empty container from the weight of the container with the dried residue to obtain the increase in weight. Then do the following:

A- Weight of clean dried container.

B- Weight of container and residue.

C- Volume of Sample (do not include rinse water) (100 ml.)

Total Dissolved Solids (mg/L) = ((B - A)/ C)X(1000 mg/g)X (1000 ml/L)

7. ALKALINITY

The alkalinity of water is its quantitative capacity to neutralize acids. Alkalinity is not a pollutant. It is a total measure of the substances in water that have "acid-neutralizing" ability. It essentially absorbs the excess H+ ions and protects the water body from fluctuations in pH. Alkalinity is often related to hardness because the main source of alkalinity is usually from carbonate rocks (limestone) which are mostly $CaCO_3$. If $CaCO_3$ actually accounts for most of the alkalinity, hardness in $CaCO_3$ is equal to alkalinity. Since hard water contains metal carbonates (mostly $CaCO_3$), it is high in alkalinity. Conversely, unless carbonate is associated with sodium or potassium which don't contribute to hardness, soft water usually has low alkalinity and little buffering capacity. So, generally, soft water is much more susceptible to fluctuations in pH from acid rains or acid contamination.

Absolutely pure water has a pH of exactly 7.0. It contains no acids, no bases, and no (zero) alkalinity. The buffered water, with a pH of 6.0, can have high alkalinity. If you add a small amount of weak acid to both water samples, the pH of the pure water will change instantly (become more acid), but the buffered water's pH won't change easily because buffers absorb the acid and keep it from "expressing itself."

Alkalinity is important for fish and aquatic life because it protects or buffers against pH changes (keeps the pH fairly constant) and makes water less vulnerable to acid rain. The main sources of natural alkalinity are rocks, which contain carbonate, bicarbonate, and hydroxide compounds. Borates, silicates, and phosphates may also contribute to alkalinity.

Limestone is rich in carbonates, so waters flowing through limestone regions generally have high alkalinity. Conversely, granite does not have minerals that contribute to alkalinity. Therefore, areas rich in granite have low alkalinity and poor buffering capacity.

At a pH of approximately 4.3 and below, we see there is no alkalinity present. There is only free mineral acidity (FMA) and dissolved carbon dioxide. (Dissolved carbon dioxide is sometimes expressed as carbonic acid, H_2CO_3.).As we increase the p^H, we see that between 4.3 and 8.3, the dissolved carbon dioxide starts to convert to bicarbonate ion. This conversion is complete at a pH of about 8.3, where only bicarbonate is present. Increasing the p^H beyond 8.3, the bicarbonate ion is converted

to carbonate ion. Conversion is nearly complete at a p^H around 10.2 with almost all the bicarbonate being converted to carbonate. Further increasing the p^H past 10.2, we start seeing measurable levels of hydroxide ions along with the carbonate ions.

Total (T) alkalinity exists when the pH is greater than 4.3. Although a pH of 7 is neutral, in water chemistry it is not this pH that separates alkalinity from acidity. In water chemistry, the pH that separates alkalinity from acidity is approximately 4.3. Waters having a pH below 4.3 are considered to have strong mineral acidity. Waters within the approximate pH range of 4.3 to 8.3 (T alkalinity to Phenolphthalein (P) alkalinity) contain bicarbonate alkalinity and weak acids such as carbonic acid (carbon dioxide in solution) may also exist. In the pH range of approximately 8.3 to 9.6 bicarbonate and carbonate alkalinity can coexist in the absence of carbon dioxide or hydroxide alkalinity. Above a pH of approximately 9.6, hydroxide alkalinity becomes measurable.

The Alkalinity of natural waters is due to the salts of carbonates, bicarbonates, borates, silicates and phosphates along with the Hydroxyl ions in the free state. The three major forms of alkalinity ranked in order of their association with high pH values are (1) hydroxide alkalinity, $[OH^-]$, (2) carbonate alkalinity, $[CO_3^{2-}]$, and (3) bicarbonate alkalinity $[HCO_3^-]$.

Their ability to react with H+ ends at pH 4.5 when both have turned into carbonic acid (H_2CO_3). In nature, bicarbonates are the major form of alkalinity because they result from the reactions of CO_2 on calcium and magnesium rocks. For all practical purposes, alkalinity due to other sources in natural waters may be ignored.

Alkalinity of waters is measured by means of titration with a standard solution of a strong acid (usually H_2SO_4) to designated p^H, and is reported in terms of equivalent $CaCO_3$. Alkalinity depends on the end-point p^H or indicator used. Either titration curve technique or colour indicators can be used for the determination. The alkalinity measurement is based on the titration curve for a hydroxide-carbonate-bicarbonate mixture,

For samples whose initial p^H is above 8.3, the titration is made in two steps. In the first step, the titration is conducted until the phenolphthalein indicator end-point is reached (i.e. p^H of about 8.3) with a colour change from pink to colourless. During this first phase, the acid added to the sample reacts with $[OH^-]$ alkalinity, if present, and $[CO_3^{2-}]$ alkalinity as follows:

$$OH^- + H^+ \rightarrow H_2O$$

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

In the second phase, the titration is continued until the methyl orange indicator end-point is reached with a colour change from yellow to red (i.e. pH of about 4.5). During this phase, the addition of acid changes the HCO_3^- ions, initially present as well as those produced by Reaction, into carbonic acid;

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$

In the above titration, the result of first step is known as "phenolphthalein (P) alkalinity" and the overall titration is known as " Total(T) alkalinity" (the amount of acid required to react with all the hydroxide, carbonates and bicarbonates in the sample) respectively.

When the pH of a sample is less than 8.3, a single titration is made to the methyl orange end point. Also for routine work, it is common that only the total alkalinity is determined.

It is possible to determine the various components of alkalinity (i.e. hydroxide, carbonate and bicarbonate fractions) from a combination of titration, pH measurements and chemical equilibrium equations.

An understanding of the buffering capacity of alkalinity can be derived. At the inflection points of pH 8.3 (phenolphthalein alkalinity) and pH 4.5 (total alkalinity), the carbonate system will react with a considerable pH change when only a small fraction of titrant is added. However, at the points where only half of the initial carbonate has been converted to bicarbonate and only half of the resultant bicarbonate has been converted to carbonic acid, considerably more titrant is required to effect a pH change. It is during these conditions that the buffering capacity is exhibited. Quantifying the alkalinity to the inflection points is a measure of this buffering capacity.

Test Method:

The Alkalinity of water is a measure of its capacity to neutralize acids, ie., ability to absorb H+ ions without significant change in pHin natural water, most of the alkalinity is caused due to CO_2. The free CO_2, dissolved in water produces carbonic acid (H_2CO_3), which dissociates into H^+ and HCO_3^-.The HCO_3^-, thus formed further dissociates in to H^+ and CO_3^{2-}. If Bicarbonate concentration is high, calcium and Magnesium concentrations are generally high.

Principle:

Alkalinity of sample can be estimated by titrating with standard Sulphuric acid. Titration or neutralizing the water sample up to pH 8.3 by Phenolphthalein Indicator,

will indicate the complete neutralization of OH- and ½ CO3 ions concentration, while Titration or neutralizing the water sample up to pH 4.5, will indicate the total alkalinity i.e., complete neutralization of OH^-, CO_3^{-2} , and HCO_3^- ions.

Chemical Reaction:

Sulfuric acid (hydrochloric acid canalso be used) reacts with the three forms of alkalinity, converting them to water or carbonic acid. If hydroxide is present, it reacts to form water:

$$2OH^- + H_2SO_4 \rightarrow 2H_2O + SO_4^{2-}$$

This conversion usually is complete at a pH of about 10. Phenolphthalein alkalinity is determined by titration to an end point pH of 8.3, which corresponds to the conversion of carbonate to bicarbonate.

$$2\ CO_3^{-2} + H_2SO_4 \rightarrow 2\ HCO_3^- + SO_4^{2-}$$

If hydroxide is present, titration to p^H 8.3 will indicate the alkalinity due to all of the hydroxide plus one-half of the carbonate. Continued titration to pH 4.5 completes the conversion of carbonate plus any bicarbonate present to carbonic acid. This value is termed Total Alkalinity.

$$2\ HCO_3^- + H_2SO_4 \rightarrow 2H_2CO_3 + SO_4^{2-}$$

Reagents:

1. Standard H2SO4, 0.02 N: Prepare 0.1N H2SO4 by diluting 3mL conc. H2SO4 to 1000mL. Standardize it against standard 0.1N Na2CO3 solution. Dilute appropriate volume of H2SO4 to 1000mL to obtain standard 0.02 H2SO4.
2. Phenolphthalein indicator: Dissolve 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add dropwise 0.02N NaOH till faint pink colour appears (pH 8.3).
3. Methyl orange indicator: Dissolve 0.5g and dilute to 1000mL with CO2 free distilled water (pH 4.3-4.5)**or**Bromo-cresol green indicator: Dissolve 0.1g bromocresolgreen, sodium salt, in 100mL distilled water (pH 4.5).

Procedure:

a. Measure and take suitable volume of sample (25 or 50 ml) in a conical flask.
b. Add 2-5 drops of Phenolphthalein Indicator, and observe the colour. If the no colour is produced, PhenolphthaleinAlkallinity is absent. If the colour turns pink, titrate with standard 0.02N H_2SO_4 solution, till the colourdisappears (i.e., indicating the pH 8.3). Note down the volume of H_2SO_4solution required (A).

c. Add 2-5 drops of Methyl Orange Indicator in the same conical flask and continue the titration with H_2SO_4 solution, till colour changes from orange yellow to pink (characteristic of pH 4.5– 4.3). Note down the Total volume of H_2SO_4solution required (B).

Calculation:

As each ml. of 0.02N H_2SO_4 = 1mg. of Alkalinity as $CaCO_3$, therefore,

a. Total Alkalinity as $CaCO_3$

= B (total volume of H2SO4required in ml.) X1000**mg./lit.**
Volume of the sample in ml.

b. Phenolphthalein Alkalinity as $CaCO_3$

= A (volume of H2SO4 required in ml.) X1000**mg./lit.**
Volume of the sample in ml.

c. Methyl Orange Alkalinity as $CaCO_3$

= B-A (volume of H2SO4 required in ml.) X1000**mg./lit.**
Volume of the sample in ml.

Once the Phenolphthalein Alkalinity and Total Alkalinity are determined, then the Alkalinity contributed by the three ions (OH^-, CO_3^{-2} , and HCO_3^-) can be calculated from the given information.

Calculations:

The various combinations of Phenolphthalein Alkalinity and Total Alkalinity(P&T) provide information of three species of alkalinity as below:

If P = 0, Total alkalinity is due to bicarbonates.

If P < T/2, Carbonate alkalinity = 2P; bicarbonate alkalinity = T – 2P.

If P > T/2 Carbonate alkalinity = 2(T - P); hydroxide alkalinity = 2P – T.

If P = T/2,Total alkalinity is due to carbonates.

If P = T, Alkalinity due to hydroxides only.

Once the Carbonate and Bicarbonate alkalinities are Known, then the conversion of levels of Carbonates (CO_3^{--}) and Bicarbonates(HCO_3^-) can be calculated, as given below.

Carbonates (CO_3^{--}) in mg./lit. = Carbonate alkalinity (in mg./lit.)/ 0.6

Bicarbonate (HCO_3^-) in mg./lit. = Bicarbonate alkalinity (in mg./lit.)/ 1.22.

8.HARDNESS

Water hardness is the traditional measure of the capacity of water to react with soap, hard water requiring considerably more soap to produce a lather. Hard water often produces a noticeable deposit of precipitate (e.g. insoluble metals, soaps or salts) in containers, including "bathtub ring". It is not caused by a single substance but by a variety of dissolved polyvalent metallic ions, predominantly calcium and magnesium cations, although other cations (e.g. aluminium, barium, iron, manganese, strontium and zinc) also contribute. Hardness in water is caused by dissolved minerals, primarily divalent cations, including calcium (Ca^{2+}), Magnesium (Mg^{2+}) iron (Fe^{2+}), strontium (Sr^{2+}), zinc (Zn^{2+}) and Manganese (Mn^{2+}). Calcium and Magnesium ions are usually the only ions present in significant concentrations; therefore, hardness is generally considered to be a measure of the Calcium and Magnesium content of water. Considerations should be given when other cations contributing to hardness are present in significant amounts. Hardness is most commonly expressed as milligrams of Calcium Carbonate equivalent per litre. Water containing calcium carbonate at concentrations below 60 mg/l is generally considered as soft; 60–120 mg/l, moderately hard; 120–180 mg/l, hard; and more than 180 mg/l, very hard. Hardness may also be discussed in terms of carbonate (temporary) and non-carbonate (permanent) hardness.

The principal natural sources of hardness in water are dissolved polyvalent metallic ions from sedimentary rocks, seepage and runoff from soils. Calcium and Magnesium, the two principal ions, are present in many sedimentary rocks, the most common being limestone and chalk.

The taste threshold for the calcium ion is in the range 100–300 mg/l, depending on the associated anion, but higher concentrations are acceptable to consumers.

The following equilibrium reaction describes the dissolving and formation of calcium carbonate :

$$CaCO_3\,(s) + CO_2\,(aq) + H_2O\,(l) \leftrightharpoons Ca^{2+}\,(aq) + 2HCO_3^-\,(aq)$$

The reaction can go in either direction. Rain containing dissolved carbon dioxide can react with calcium carbonate and carry calcium ions away with it. The calcium carbonate may be re-deposited as calcite as the carbon dioxide is lost to atmosphere.

Temporary hardness is a type of water hardness caused by the presence of dissolved bicarbonate minerals (calcium bicarbonate and magnesium bicarbonate). When dissolved in water, these minerals yield calcium &magnesium cations (Ca^{2+},Mg^{2+})

and carbonate & bicarbonate anions (CO_3^{2-}, HCO_3^-). However, Permanent hardness is caused by sulfate and chloridecompounds. The "temporary" hardness can be reduced either by boiling the water, or by the addition of lime (calcium hydroxide). Boiling promotes the formation of carbonate from the bicarbonate and precipitate as calcium and Magnesium carbonates out of solution, leaving water that is softer upon cooling.

Permanent hardness is hardness (mineral content) that cannot be removed by boiling. It is usually caused by the presence of calcium sulfate and/or Magnesium sulfates in the water, which do not precipitate out as the temperature increases. Ions causing permanent hardness of water can be removed using a water softener, or ion exchange column.

Total Hardness = Calcium Hardness + Magnesium Hardness

The calcium and magnesium hardness is the concentration of calcium and magnesium ions expressed as equivalent of calcium carbonate. The presence of these cations, makes the water Hard.

Total water hardness expressed as equivalent of $CaCO_3$ can be calculated with the following formula:

Total Hardness ($CaCO_3$) = $2.5(Ca^{2+}) + 4.1(Mg^{2+})$

Health effects:

Exposure to hard water has been suggested to be a risk factor that could exacerbate eczema. The environment plays an important part in the etiology of atopic eczema, but specific causes are unknown. Numerous factors have been associated with eczema flare-up, including dust, nylon, shampoo, sweating, swimming and wool A suggested explanation relative to hard water is that increased soap usage in hard water results in metal or soap salt residues on the skin (or on clothes) that are not easily rinsed off and that lead to contact irritation.

Corrosion and scaling:

Depending upon interactions with other factors, such as pH and alkalinity, hard water can cause,

- Increased soap consumption
- Scale deposition in the water distribution system, as well as in heated water applications where insoluble metal carbonates are formed
- Reducing the efficiency of heat exchangers.

Excessively hard water can also have corrosion tendencies. Soft water that is not stabilized has a great tendency to cause corrosion of metal surfaces and pipes in the distribution system, resulting in the presence of certain heavy metals, such as cadmium, copper, lead and zinc, in drinking-water. Corrosion of heavy metals can be associated with health risks (from leachates such as lead, copper and other metals) and reduced lifespan of the distribution network. Soft or softened waters do have the benefit of minimal scaling and therefore allow more efficient heat transfer in exchangers and probably longer life of hot water heaters.

Units of Hardness:

The various alternative units represent an equivalent mass of calcium oxide (CaO) or calcium carbonate ($CaCO_3$) that, when dissolved in a unit volume of pure water, would result in the same total molar concentration of Mg^{2+} and Ca^{2+}. The different conversion factors arise from the fact that equivalent masses of calcium oxide and calcium carbonates differ, and that different mass and volume units are used. The units are as follows:

- *Parts per million (ppm)* is usually defined as 1 mg/L $CaCO_3$ It is equivalent to **mg/L** without chemical compound specified,

Test Method:

Principle:

Hardness in water can be determined quickly by titration and the use of color indicators. By proper choice of p^H, total hardness (Ca2+ and Mg2+) or the portion contributed by calcium and magnesium individually can be measured. The traditional test for hardness involves P^H adjustment to 10 with an ammonium buffer, addition of Eriochrome Black T indicator [1-(1- hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid] and then titration with Na_2EDTA (Ethylene-Diamine-Tetraacetic acid of Disodium salt) solution.

Disodium salt of Ethylene-Diamine tetra acetic acid (Na_2 EDTA) forms chelated soluble complexes when added to a solution of certain metal cations. If a small amount of a dye such a Erichrome Black T (EBT) is added to an aqueous solution containing calcium and Magnesium ions, at a pH of 10.0+/- 0.1, the solution becomes wine red coloured. If EDTA is then added as a titrant, the Calcium and Magnesium ions will form soluble complexes with the EDTA. After sufficient EDTA has been added , all the Calcium and Magnesium ions will be complexed, and the colour of the solution will changed sharply from wine red to blue, which indicates the end-point of the titration.

Magnesium ion must be present to yield a satisfactory end point in the titration. A small amount of complexometrically neutral magnesium salt of EDTA is therefore added to the buffer. The sharpness of the end-point increases with increase in the pH. However, the pH can not be increased beyond 10.1, because of the danger of precipitating the $CaCO_3$ or $Mg(OH)_2$. A limit of 5 minutes is set for the duration of the titration to minimize the tendency of $CaCO_3$ precipitation.

Interference:

Some metal ions interfere with this procedure by causing the fading or indistinct end points. This interference is reduced by the addition of certain inhibitors to the water sample before titration with EDTA. For eg. Hydroxylamine Hydrochloride in 95% ethyl or Propyl Alcohol can be used where interference caused by Aluminum (up to 20 mg./lit), Copper (up to 0.3 mg./lit), Iron (up to 20 mg./lit) and Nickel ((up to 2 mg./lit).

Reagents:

1. Buffer solution: Dissolve 16.9 g NH4Cl in 143mL NH4OH. Add 1.25 g magnesium salt of EDTA to obtain sharp change in colour of indicator and dilute to 250mL. If magnesium salt of EDTA is not available, dissolve 1.179 gms. of Di sodium salt of EDTA (AR grade) and 780 mg MgSO4.7H2O or 644 mg MgCl2.6H2O in 50mL distilled water. Add this to above solution of NH4Cl in NH4OH and dilute to 250mL.
2. Inhibitor: Dissolve 4.5g hydroxylamine hydrochloride in 100mL 95% ethyl alcohol or isopropyl alcohol.
3. Eriochrome black T indicator: Mix 0.5g dye with 100g NaCl to prepare dry powder.
4. Murexide indicator: Prepare a ground mixture of 200mg of murexide with 100g of solid NaCl.
5. Sodium hydroxide 2N: Dissolve 80g NaOH and dilute to 1000mL.
6. Standard EDTA solution 0.01 M: Dissolve 3.723 g EDTA sodium salt and dilute to 100mL. Standardise against standard Calcium solution 1mL = 1 mg CaCO3.
7. Standard calcium solution: Weigh accurately 1g CaCO3 (AR grade) and transfer to 250mL conical flask. Place funnel in the neck of a flask and add 1+1 HCl till CaCO3 dissolves completely. Add 200mL distilled water and boil for 20-30 minutes to expel CO2. Cool and add few drops of methyl red indictor. Add 8N

NH4OH drop-wise till intermediate orange colour develops. Dilute to 1000mL to obtain 1mL = 1mg CaCO3.

Procedure:

a. Measure and take suitable volume of sample (25 or 50 ml) in a conical flask.
b. Add 1-2 ml. of buffer solution to raise the pH of the sample to 10.0, followed by 1 ml. of Hydroxylamaine Hydrochloride Inhibitor.
c. Add a pinch of Erichrome Black T (EBT) indicator. The solution turns in to wine red colour. Then titrate with Standard EDTA (0.01 M) Solution till the wine red colour changes to blue. Note down the volume of the EDTA consumed (A).
d. Run a reagent blank. Note down the volume of the EDTA (B).
e. For natural waters of low hardness, take a larger sample volume, i.e., 100-200 ml. for titration and proportionately larger amount of buffer, inhibitor and indicator. Apply blank corrections for computing the results.

Calculation:

As each ml. of 0.01M EDTA solution = 1mg. of Hardness as $CaCO_3$, therefore,

Total Hardness as $CaCO_3$ **mg./lit.**

$$= \frac{\text{Volume of EDTArequired in ml. by the sample (A-B)) X1000}}{\text{Volume of the sample in ml.}}$$

Carbonate Hardness: It is the hardness caused bycarbonates and Bicarbonates of metals other than alkali metals.

Non Carbonate Hardness: It is the hardness caused by salts other than carbonates and Bicarbonates of metals.

Calculation of Carbonate hardness and non carbonate hardness:

When both Total alkalinity and Total Hardness are expressed in terms of $CaCO_3$, the below relationships holds good.

a. When Total Alkalinity < Total Hardness,
   Carbonate hardness = Total Alkalinity.
   Non Carbonate Hardness = Total Hardness – Carbonate hardness.
b. When Total Alkalinity >/= Total Hardness,
   Carbonate hardness = Total Hardness.
   Non Carbonate Hardness is absent.

Interferences:

Some metal ions interfere by causing fading or indistinct end points or by stoichiometric of EDTA but can be reduced by addition of inhibitors. Suspended or colloidal organic matter may also interfere with the end point. This interference can be eliminated by evaporating 50mL sample to dryness on a steam bath and then heating in a muffle furnace at 550°C. Residue may be dissolved in 20mL of 1N hydrochloric acid and on neutralization to pH 7 with 1N sodium hydroxide, volume be made to 50mL with distilled water. Run a reagent blank following the same procedure.

9. CALCIUM

Principle:

When EDTA is added to a water sample containing both Calcium and Magnesium, it combines first with Calcium. Hence Calcium can be determined directly by titrating with EDTA, when the pH is raised sufficiently high so that Magnesium is completely precipitated as Mg(OH)2 and a suitable indicator is used such that it combined with Calcium only. Murexide (Ammonium Purpurate) indicator give a colour change when all the calcium has been completely complexed with EDTA at pH 12 to 13.

Reagents:

1. Standard Ethylene-Diamine tetra acetic acid or its sodium salts (Na_2 EDTA) of 0.01M.
2. Sodium Hydroxide solution (8%) to raise the pH of the sample Solution to pH 12-13.
3. Murexide (Ammonium Purpurate) indicator: This indicator changes the colourfrom pink to purple colour at the end point of the titration.

Procedure:

a. Measure and take suitable volume of sample (25 or 50 ml) in a conical flask.

b. Add 1 ml. of Sodium Hydroxide solution to raise the pH of the sample to 12-13.

c. Add a pinch of Murexide (Ammonium Purpurate) indicator. The solution turns in to pink colour. Then titrate with Standard EDTA (0.01M) Solution until the pink colour changes to purple. Note down the volume of the EDTA consumed.

(Note: Titration should be completed within 5 minutes after addition of NaOH solution)

Calculation:

As each ml. of 0.01M EDTA solution = 1mg. of Calcium as CaCO3, therefore,

Calcium Hardness as CaCO3

$$= \frac{\text{(volume of EDTArequired in ml.) X1000mg./lit.}}{\text{Volume of the sample in ml.}}$$

Calcium as Ca in mg./lit = Calcium Hardness as CaCO3 X 0.4005

10. MAGNESIUM

Magnesium can be determined by calculating the difference between the total hardness and the calcium hardness of the sample (when both are expressed as CaCO3).This yields the value of magnesium hardness as $CaCO_3$ in mg./lit.

Magnesium hardness as $CaCO_3$

= Total Hardness as $CaCO_3$ - Calcium Hardness as $CaCO_3$.

Magnesium as Mg in mg/lit = Magnesium hardness as $CaCO_3$ X0.2431.

11. CHLORIDE

Introduction

The presence of chloride in natural waters can be attributed to dissolution of salt deposits, discharges of effluents from chemical industries, oil well operations and seawater intrusion in coastal areas. Each of these sources may result in local contamination of both surface water and groundwater. The salty taste produces by chloride depends on the chemical composition of the water. A concentration of 250mg/L may be detectable in some waters containing sodium ions. On the other hand, the typical salty taste may be absent in water containing 1000 mg./L chloride when calcium and magnesium ions are predominant. High chloride content may harm pipes and structures as well as agricultural plants.

The taste threshold of the chloride anion in water is dependent on the associated cation. Taste thresholds for sodium chloride and calcium chloride in water are in the range 200–300 mg/litre.

Chloride increases the electrical conductivity of water and thus increases its corrosive property. In metal pipes, chloride reacts with metal ions to form soluble salts, thus increasing levels of metals in drinking-water. In lead pipes, a protective oxide layer is built up, but chloride enhances galvanic corrosion.

Argentometric method

This method is used for the analysis of the chloride ion present in the natural water. The Mercurimetric method is recommended when an accurate determination of chloride is required, particularly at low concentrations. The potentiometric method is suitable only when the sample is coloured or turbid, argentometric method is the simplest method of choice for variety of samples.

1. Principle

The amount of *chloride in water* can be simply determined by titrating the collected water sample with silver nitrate solution by using potassium chromate indicator. The reaction is quantitative. The $AgNO_3$ reacts with chloride ion in a 1.1 ratio. The result is expressed as mg./lit.

The end point of the titration takes place when all the chloride ions reacts and precipitated. Then slightly extra silver ions react with the chromate ions and form a

brownish-red precipitate of silver chromate. The solubility product of silver chromate exceeded in the presence of additional silver ions, and then the precipitation occurs.

The chemical reactions involved in this method are given below:

1. $Ag^+ + Cl^-$:AgCl (White precipitate)
2. $2Ag^+ + CrO4^{-2}$:Ag_2CrO_4 (Red precipitate)

2. Reagents and standards

a. Potassium chromate indicator: Dissolve 50g K_2CrO4 in distilled water. Add $AgNO_3$ till definite red precipitate is formed. Allow to stand for 12 hrs. Filter and dilute to 1000mL.

b. Silver nitrate (0.0141N) : Dissolve 2.395g $AgNO_3$ and dilute to 1000mL. Standardise against NaCl(0.0141N).

1mL of 0.0141N $AgNO_3$ = 0.5 mg Cl^-.

c. Sodium chloride, 0.0141N: Dissolve 824.1mg NaCl (dried at 40°C) and dilute to 1000mL; 1mL = 0.5 mg Cl^-.

d. Special reagent to remove colour and turbidity: Dissolve 125g $AlK(SO_4)_2.12H_2O$ or $AlNH_4(SO_4)_2.12H_2O$ and dilute to 1000mL. Warm to 60°C and add 55mL conc. NH_4OH slowly. Let the solution stand for 1 hour. Transfer to a large bottle and wash precipitate by successive addition with thorough mixing and decanting with distilled water. When freshly prepared, a suspension occupies a volume of approximately 1L.

3. Calibration

The silver nitrate solution should be standardised against sodium chloride solution of 0.0141N. It gives the strength of silver nitrate solution 1mL = 0.5mg chlorides as Cl^-.

4. Procedure

a. Take 50mL well mixed sample adjusted to pH 7.0-8.0and add 1.0 mL $K2CrO_4$.

b. Titrate with standard $AgNO_3$solution till $AgCrO_4$ starts precipitating as pale red precipitate

c. Standardise $AgNO_3$ against standard NaCl

d. For better accuracy titrate distilled water (50mL) in the same way to establish reagent blank. A blank of 0.2 to 0.3mL is usual.

5. Calculation

Chloride mg/L as Cl- = (A –B) x N x 35.45 x 1000 / mL sample

where,A = ml. $AgNO_3$ required for sample, B = ml. $AgNO_3$required for blank, and

N = Normality of $AgNO_3$ used.

6. Interferences

Bromide, iodide and cyanide are measured as equivalents of chloride ions.Sufficient concentrations of thiosulphate, thiocyanate, cyanide, sulphate and sulphide, interfere seriously with the determination of chloride.

9. Limitation

The *Mohr method* for *determination of chloride* must be perform between the pH levels 6.5-9.0. It is better to carry out between the pH ranges 7-8. At upper pH level, the silver ions react with hydroxide ions and precipitated as silver hydroxide. In contrast, at lower pH level, potassium chromate may be converted into potassium dichromate ($K_2Cr_2O_7$) and mask the end point. Consequently, accurate result cannot be obtained. If the water sample is acidic, then gravimetric method or volhard's method is appropriate.

$Ag^+_{(aq)} + OH^-_{(aq)} \rightarrow Ag(OH)_{(s)}$

$CrO_4^{2-}{}_{(aq)} \rightarrow Cr_2O_7^{2-}{}_{(aq)}$

12. FLUORIDE (F-)

Introduction:

Among the many methods suggested for the determination fluoride ion in water, the Colorimetric method (SPANDS) and the ion selective electrode method are the most satisfactory and applicable to variety of samples. Because all of the Colorimetric methods are subject to errors due to presence of interfering ions, it may be necessary to distill the sample before making the fluoride estimation.

A. Ion selective electrode method

The fluoride electrode is an ion-selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other. The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. Adding an appropriate buffer provides a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration of Fluoride insolution.

Principle

When the fluoride electrode is dipped in sample whose concentration is to be measured, a potential is established by the presence of fluoride ions across the lanthanum fluoride crystal, which is measured by a device called Ion Selective Electrode Meter or by any modern pH meter having an expanded millivolt scale. The fluoride ion selective electrode can be used to measure the activity or concentration of fluoride in aqueous sample by use of an appropriate calibration curve. However, fluoride activity depends on the total ionic strength of the sample, the electrode does not respond to the bound or complexed fluoride. Addition of a buffer solution of high total ionic strength containing a chelate to complex aluminium preferentiality overcomes these difficulties.

Apparatus and equipment

a. Ion meter (field/laboratory mode) or pH/mV meter for precision laboratory measurements

b. Reference electrode (calomel electrode)

c. Fluoride-sensitive electrodes

d. Magnetic stirrer

e. Plastic lab ware (samples and standards should always be stored in plastic containers as fluoride reacts with glass).

Reagents and standards

a. Stock fluoride solution: Dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100 µg F^-.

Standard fluoride solution: Dilute stock solution 10 times with distilled water to obtain 1mL = 10µg F^-.

c. Total Ionic Strength Adjustment Buffer :

It provide a constant background ionic strength, decomplexes fluoride ions and adjusts the pH of the solution.

Preparation of low level TISAB:

For measuring the Fluoride in samples which contains less than0.4 ppm of Fluoride and Fluoride complexing agents such as iron and aluminum are notpresent.

Place approximately 500mL distilled water in a 1L beaker. Add 57mL glacial acetic acid, 58 gms., of reagent grade Sodium Chloride (NaCl) and 4 gms. of 1, 2-cyclohexylenediamine tetra acetic acid (CDTA). Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125mL) with stirring, until pH is between 5 to 5.5. Transfer to a 1L volumetric flask and make up the volume to the mark.

ii) Preparation of Concentrated TISAB :

Take 200 ml. of Con. HCL in 1Lit.beaker. Add 394 gms of Ammonium Acetate and 17.8 gms of CDTA. Dissolve them and make up to 1 lit. with distilled water and adjust the pH in between 5-5.5 by the addition of 6N NaOH solution.

Sample collection, preservation and storage

Polyethylene bottles are preferred for collecting and storing samples for fluoride analysis.

Calibration

Take 50mL of each 1ppm and 10ppm fluoride standard. Add equal volume , i.e., 50mL, of Low Level TISAB (or 10% i.e., 5mL of concentrated TISAB) and calibrate the instrument.

Procedure

a. For connecting the electrodes to meter and for further operation of the instrument follow the instruction manual supplied by the manufacturer.

b. Check the electrode slope with the ion meter (59.16mV for monovalent ions and 29.58mV for divalent ions at 25°C).

c. Transfer 50mL of sample to a 150mL plastic beaker. Add equal volume of Low level TISAB or 5m of concentrated TISAB (10%).

d. Rinse electrode, blot dry and place in the sample. Stir thoroughly and note down the steady reading on the meter.

e. Recalibrate every 1 or 2 hours.

f. Direct measurement is a simple procedure for measuring a large number of samples. The temperature of samples and standard should be the same and the ionic strength of standard and samples should be made the same by addition of TISAB to all solutions.

g. Direct measurement results can be verified by a known addition procedure. The known addition procedure involves adding a standard of known concentration to a sample solution.

Interferences

Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride, and complexing species. However, CDTA (cyclohexylenediaminetetraacetic acid), a component of the buffer, preferentially will complex interfering cations and release free fluoride ions. Concentrations of aluminum, the most common interference, up to 3.0 mg/L can be complexed preferentially. In acid solution, F^- forms a poorly ionized HF -HF complex but the buffer maintains a pH above 5 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. At the pH maintained by the buffer, no hydroxide interference occurs.

B. Sodium 2-(parasulphophenylazo)-1,8-dihydoxy-3,6-naphthalene disulphonate (SPADNS) method

This method is used for estimation of fluoride in natural water in the concentration range 0-1.4 mg/L.

Principle

Under acidic condition fluoride (F-) reacts with zirconium-SPANDS- dye-lake, dissociating a portion of it into a colourless complex anion (Zr F6 -) and the dye. As the amount of fluoride increases, the colour produced becomes progressively lighter and hence it obeys Beer's law in a reverse manner.

The chemical reaction involved in the method is given below:

(Zr-SPANDS-lake) + 6 F^- = (SPANDS Red bleached) + ZrF_6^-

12. Apparatus and equipment

a. Distillation apparatus

b. Colorimeter for use at 570nm

c. Nessler tubes cap. 100mL.

d. Pipette

13. Reagents and standards

a. Sulphuric acid: H2SO4, Conc.

b. Silver sulphate: Ag2SO4, crystals.

c. SPANDS solution: dissolve 958 mg SPANDS in distilled water and dilute to 500mL.This solution is stable for atleast 1 year if protected from direct sunlight.

d. Zirconyl acid reagent: dissolve 133mg ZrOCl2.8H2O in 25mL water. Add 350mL conc. HCl and dilute to 500mL.

e. AcidZirconyl-SPANDS reagent: Mix equal volume of 'c' and'd' to produce a singie reagent. Protect from direct light. This combined reagent is stable for at least 2 years.

f. Reference solution: add 10mL SPANDS solution to 100mL distilled water. Dilute 7mL conc. HCl to 10mL and add to dilute SPANDS solution. Use this solution for setting zero of spectrophotometer. It is stable for 1 year. Alternatively use a prepared standard of 0mg F-/L as a reference.

g. Sodium Arsenite solution: Dissolve 5.0g NaAsO2 and dilute to 1000mL. (Caution: Toxic-avoid ingestion)

h. Stock fluoride solution: dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100µg F-.

i. Standard fluoride solution: dilute stock solution 10 times with distilled water to obtain 1 mL = 10 µg F-.

Preparation of standard curve:

Prepare fluoride standards in the range of 0 to 1.4 mg/L by diluting appropriate quantities of the standard fluoride solution to 50mL with distilled water. Pipette out 5.00mL each of SPANDS solution and Zirconyl-acid reagent, or 10mL of the mixed acid-Zirconyl-SPANDS reagent. Add to each standard and mix well. Avoid contamination during the process. Set the photometer to zero absorbance with the reference solution and obtain the absorbance readings of the standards immediately. Plot a curve of the fluoride-absorbance relationship. If the standard contains residual chlorine remove it by addition of 1 drop (0.05 mL) NaAsO2 solution/ 0.1 mg residual chlorine and mix. (Sodium arsenite concentrationsof 1300 mg/L produce an error of 0.1 mg/L at 1.0 mg F–/L.)

Colour development

a. Prepare standard curve in the range 0.0 to 1.40 mg/L by diluting appropriate volume of standard fluoride solution to 50mL in Nessler tubes.

b. Add 10.0mL acid Zirconyl-SPANDS reagent to all the samples, mix well and read absorbance of bleached colour at 570nm using reference solution for setting zero absorbance.

c. Plot concentration vs per cent of absorbance.

d. Calculate mg F-/L present in the sample using standard curve.

Calculation

µg/L,F = (A / mL sample) x (B/C)

where A = µg F- determined photometrically. The ration B/C applies only when a sample is diluted to a volume B, and portion C taken from it for colour development.

Interferences

Alkalinity 5000mg/L, aluminium 0.1 mg/L, chlorides 72000mg/L, Fe 10 mg/L, Phosphate 16mg/L, Sulphate 200mg/L, and hexametaphosphate 1.0mg/L interfere in the bleaching action. Residual chlorine interferes in bleaching action. In presence of interfering radicals distillation of sample is recommended. Sodium Arsenite is used in the SPADNS method as a reducing agent to prevent interference from chlorine and other oxidants that are typically present in drinking water.

13. NITROGEN AMMONIA

INTRODUCTION:

Ammonia is produced by the microbiological degradation of organic nitrogeneous matter. It appears, therefore in many ground waters as well surface waters.Concentrations of ammonia above certain level in water polluted either due to sewerage or industrial waste. The portions of the two forms of ammonia nitrogen in surface water depends on pH.

pH	6	7	8	9	10	11
$\%NH_3$	0	1	4	25	78	96
$\%NH_4$	100	99	96	75	22	4

For accurate results , it is generally preferable to distill off ammonia from the sample and absorb in boric acid. It is then determined either by titration or colorimetrically using Nessler reagent. Direct Nesslerisation of the sample is quicker depending upon interference.

Nesslerisation of Method:

Principle:

Ammonia produces a yellow coloured compound when reacts with alkaline Nessler reagent, provided the smaple is calrified properly. Pretreatment with ZnSO4 and NaOH precipitates Ca,Fe, Mg and sulfide and removes turbidity and apparent colour. Addition of EDTA (beforeNessler reagent) or Rochellesalt solution prevents precipitation of residual Ca and Mg in the presence of alkaline Nessler reagent .

The chemical reaction :

$$2K_2HgI_4 + NH_3 + 3KOH \rightarrow NH_2Hg_2IO + 7KI + 2H_2O$$

Apparatus:

a. Spectrophotometer having range of 300 to 700 nm,
b. Nessler tubes or 100 ml. capacity volumetric flasks.

Reagents:

a. Zinc Sulfate: dissolve 10 gms. of ZnSO4.7H2O in a distilled water and dilute to 100 ml.
b. Sodium Hydroxide (6N): Dissolve 24 gms.ofNaOH and dilute to 100 ml.

c. EDTA reagent: Dissolve 50 gms of EDTA in 60 ml. water containing 10 gms. ofNaOH. Cool and dilute to 100 ml.

d. Rochelle Salt solution : Dissolve 50 gms.of Potassium sodium tartarate in 100 ml. Remove ammonia by boiling of 30 ml. solution, cool and dilute to 100ml.

e. Nessler Reagent: Mix well 100 gms of HgI2 and 70 gms. of KI. Dissolve in small quantity of water. Add this mixure to a cooled solution of 160 gms. ofNaOH in 500 ml. of water. Dilute to 1000 ml. Keep over night. Store supernatant in coloured bottle.

f. Standard ammonia Solution: Take 3.819 gms. of NH_4Cl, dried at 100 °C, dissolve in distilled water and dilute to 1000 ml. Dilute 10 ml. of the solution to 1000 ml. 1ml. = 10µg N or 12.2 µg NH_3.

Calibration & Standardization:

Prepare calibration curve using suitable aliquots of standard solution in the range of 5 to 120 µg/ 100 ml. for reference following the same procedure as a to e, but using standard solution in place of sample.

Procedure:

a. Take 100 ml. of sample. Add 1ml. of ZnSO4 solution and 0.4 to 0.5 ml. of NaOH to obtain the pH of 10.5. Allow to settle and filter the supernatant through 42 No. Whatman filter paper.

b. Take suitable aliquot of the sample.

c. Add 3drops of Rochelle salt solution or 1 drop of EDTA Solution and mix well.

d. Add 3 ml. of Nessler reagent. Make up to 100 ml.

e. Mix well and read percent transmission after 10 minutes at 410 nm, using blank prepared in the same way by taking distilled water, instead of sample.

Calculation: The concentration may be obtained directly from standard graph.

Interferences: Colour , turbidity, Calcium & Magnesium salts and Iron in the sample constitute the prime sources of interferences.

14. NITROGEN (NITRATE) (NO3-)

Introduction:

Determination of nitrate (NO_3-) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present and the limited concentration ranges of the various techniques. Nitrate is the most highly oxidised form of nitrogen compounds commonly present in natural waters. Significant sources of nitrate are chemical fertilizers, decayed vegetable and animal matter, domestic effluents, sewage sludge disposal to land, industrial discharge, and leachates from refuse dumps and atmospheric washout. Depending on the situation, these sources can contaminate streams, rivers, lakes and ground water. Unpolluted natural water contains minute amounts of nitrate. Excessive concentration in drinking water is considered hazardous for infants because of its reduction to nitrite in intestinal track causing methemoglobinaemia. In surface water, nitrate is a nutrient taken up by plants and converted into cell protein. The growth stimulation of plants, especially of algae may cause objectionable eutrophication.

A. UV spectrophotometer method

The method is useful for the water free from organic contaminants. Measurement of the ultraviolet absorption at 220nm enables rapid determination of nitrate. The nitrate calibration curve follows Beer's law upto 11mg/Las Nitrogen. Acidification with 1N hydrochloric acid is designed to present interference from hydroxide or concentrations up to 1,000mg/L as $CaCO_3$. Chloride has no effect on the determination. Minimum detectable concentration is 40µg/L NO_3 -N.

Principle

Nitrate is determined by measuring the absorbance at 220nm in sample containing 1mL of hydrochloric acid (1N) in 100mL sample. The concentration is calculated from graph from standard nitrate solution in range 1-11mg/L as N.

Apparatus and equipment

a. Spectrophotometer, for use at 220nm and 275nm with matched silica cells of 1cm or longer light path.

b. Filter: One of the following is required.

i) Membrane filter: 0.45µm membrane filter, and appropriate filter assemble

ii) Paper: Acid-washed, ashless hard-finish filter paper sufficiently retentive for fine precipitates.

c. Nessler tubes, 50mL, short form.

Reagents and standards

a. Redistilled water: use redistilled water for the preparation of all solutions and dilutions.

b. Stock nitrate solution: dissolve 721.8mg anhydrous potassium nitrate and dilute to 1000ml with distilled water. 1mL = 100 µg N = 443µg NO3-.

c. Standard nitrate solution: dilute 100mL stock nitrate solution to 1000mL with distilled water. 1mL = 10µg NO3 N = 44.3µg NO3.

d. Hydrochloric acid solution: HCl, 1N.

e. Aluminium hydroxide suspension: dissolve 125g potash alum in 1000mL distilled water. Warm to 60°C, add 55-60mL NH4OH and allow to stand for 1h. Decant the supernatant and wash the precipitate a number of times till it is free from Cl, NO2 and NO_3. Finally after setting, decant off as much clean liquid as possible, leaving only the concentrated suspension.

Calibration

Prepare nitrate calibration standards in the range 0 to 350µg N by diluting 1, 2, 4, 7.....35mL of the standard nitrate solution to 50mL. Treat the nitrate standards in the same manner as the samples.

Procedure

Read the absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain the nitrate reading and, if necessary, a wavelength of 275nm to obtain interference due to dissolved organic matter.

Calculation

For correction for dissolved organic matter, subtract 2 times the reading at 275nm from the reading at 220nm to obtain the absorbance due to nitrate. Convert this absorbance value into equivalent nitrate by reading the nitrate value from a standard calibration curve.

Nitrate N, mg/L = mg nitrate-N / mL of sample

NO3, mg/L = Nitrate N mg/L x 4.43

Precision and Bias

Because dissolved organic matter may also absorb at 220nm and nitrate does not absorb at 275nm a second measurement can be made at 275nm to correct the nitrate

value. The extent of this empirical correction is related to the nature and concentration of the organic matter and may vary from one water to another. Filtration of the sample is intended to remove possible interference from suspended particles. Analyse the sample in duplicate for quality assurance and run 1-2 standards for quality control.

Interferences

Dissolved organic matter, nitrite, hexavalent chromium and surfactants are interferences. The latter three substances may be compensated for by independent analysis of their concentrations and preparation of individual correction curves. Organic matter can cause a positive but variable interference. The degree of interference depends on the nature and concentration of the organic matter in the sample. Clean all glassware thoroughly and rinse to reduce the error that might result from streaks or particles on the outside of the curves, as well as traces of surfactants or dichromate cleaning solution that might adhere on the interior glass surfaces.

Treat coloured samples with aluminium hydroxide suspension or dilute to minimisecolour interference.

If the sample has a high colour or is known to contain organic interferences, add 4mL $Al2(OH)_3$ suspension/100mL sample in an Erlenmeyer flask. Swirl to mix and settle for 5 minutes. Filter through a 0.45µm membrane filter previously washed with about 200mL distilled water. To 50mL clear filtered sample, add 1mL (1N), HCl and mix thoroughly.

15. NITROGEN (NITRITE) (NO2-)

Introduction:

Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. As an intermediate stage in the nitrogen cycle, it is unstable. A usual concentration in natural water is in the range of some tenths of mg/L. Higher concentrations are present in industrial wastes, sewage and

in biologically purified effluents and in polluted streams. In chlorinated supplies, levels of nitrite are often less than the limit of detection, i.e. 0.005mg/L NO2

-N but high levels may occur in un chlorinated water. Very high nitrite levels are usually associated with water of unsatisfactory microbiological activity.

A. Colorimetric method

Principle

Nitrite (NO2-) is determined through formation of a reddish purple azodye at pH 2.0-2.5 by coupling diazotized sulphanilamide with N-(1-Naphthyl) ethylenediaminedihydrochloride (NEDdihydrochloride).

The method is applicable to 1µg NO2 - N/L. The colour system obeys Beer's law up to 180µg N/L with 1cm light path at 543nm. The chemical reaction involved in the method is given below:

N2Cl Azodye

1. H2SO4 + HNO2 + HCl ---------->Diazonium salt + 2H2O

2. Diazotisation Reaction:

R – NH2 + NO2 - + 2H+ ---------->R --N = N + 2H2O
(Sulphanilamide)

3. Coupling Reaction

R –N = N + -R' --------> R –N = N -- R' + H

+[1-N-(1-Napthyl) Ethylenediamine di hydrochloride]

Where, R is SO2.NH2 and R' is –NH –CH2 –CH2-NH2

Apparatus and equipment

1. Colorimeter or spectro-photometer that can be operated at 543nm.
2. Nessler tubes or 100mL capacity volumetric flask.

Reagents and standards

1. Sulphanilamide reagent: dissolve 5g sulphanilamide in a mixture of 50mL conc. HCl and about 300mL water. Dilute to 500mL with water. This solution is stables for many months.

2. NED-dihydrochloride solution: dissolve in 500mg N-(1-Naphthyl)-ethylenediaminedihydrochloride in 500mL water. Store in a dark bottle and replace monthly or immediately when it develops a strong brown colour.

3. Sodium oxalate (0.05N): dissolve 3.35g $Na_2C_2O_4$ in water dilute to 1000mL.

4. Stock nitrite solution: dissolve 1.2320 sodium nitrite ($NaNO_2$) in water and dilute to 1000mL; 1mL = 250µg N.

5. Standard nitrite solution: Dilute appropriate aliquot of stock nitrite solution to 1000mL with distilled water so that 1mL = 0.5 µg N in the solution.

Sample collection, preservation and storage

Most reliable are obtained in fresh sample as nitrite is an intermediate oxidation state of nitrogen, in oxidation of ammonia to nitrate and reduction of nitrate. Sample can be preserved by addition of $HgCl_2$ for a short period, if necessary. Acid preservation is not used in any case.

Procedure

1. If sample contains suspended solids, filter through a 0.45 mm pore diameter membrane filter.

2. To 50mL clear sample neutralized to pH 7 or to a portion diluted to 50mL, add 1.0 ml sulfanilamide solution. Let reagent react for 2 to 8 min.

3. Add 1.0mL NED dihydrochloride solution and mix immediately. Measure absorbance after 10 min but before 2 h at 543 nm.

4. Prepare blank in the same way substituting water for the sample.

Calibration

1. Prepare calibration curve by pipetting suitable volume of standard nitrite solution in the range 0-180µg NO_2 –N/L using 1cm light path for reference.

2. Run parallel check frequently against nitrite standards preferably in the concentration range of sample. Re-determine complete calibration curve after preparing new reagents. Compute concentration of sample from calibration curves.

Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55 and 1.04mg NO_3^- + NO_2^- - N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01 respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55 and 1.04mg NO_3^- + NO_2^- -

N/L, the recoveries were 100%, 102% and 100% respectively.

Interferences

Chlorine in the samples or nitrogen trichloride which normally coexists with NO_2^- produces a false colour. This can be minimised by addition of the NED dihydrochloride reagent followed by the suphanilamide acid reagent. An orange colour still may result when a substantial NCl_3 concentration is present. Under such circumstances, check for free Chlorine and NCl_3 residuals. NO_2 determinations should be carried out in filtered, turbidity-free sample.

16. SULPHATE (SO4--)

Introduction:

Sulphate ions usually occur in natural waters. Many sulphate compounds are readily soluble in water. Most of them originate from the oxidation of sulphide ores, solution of gypsum and anhydrite, the presence of shales, particularly those rich in organic compounds, and the existence of industrial wastes. Atmospheric sulphur dioxide formed by the combustion of fossil fuels and emitted by the metallurgical roasting processes may also contribute to the sulphate compounds in water. Sulphur trioxide (SO_3) produced by the photolytic oxidation of sulphur dioxide,dissolves inatmospheric water vapour to form traces of sulphuric acid and precipitates as acid rain or snow. Sulphur-bearing mineral are common in most sedimentary rocks. In the weathering process gypsum (calcium sulphate) gets dissolved in water.Sulphur from sulphide minerals get partly oxidised, giving rise to a soluble forms of sulphate that is carried away bywater. In humid region, sulphate is readily leached from the zone of weathering by infiltrating waters and surface run off but in semiarid and arid regions the soluble salts may accumulate within a few tens of feet of land surface. Under such situations, sulphate concentration in shallow ground water may exceed 5000mg/L and gradually decrease with depth.

Ingestion of water containing high concentration of sulphate can have a laxative effect, which is enhanced when sulphate is consumed in combination with magnesium. Water containing magnesium sulphate at levels about 1000 mg/L acts as a purgative in human adults. Taste threshold concentrations for the most prevalent sulphate salts are 200-500mg/L for sodium sulphate,

250- 900mg/L for calcium sulphate, and 400-600mg/L for magnesium sulphate. Essentially on the basis of above values, which are also allied to the cathartic effect of sulphate, a guidelines value of 400mg/L is proposed. Sulphates cause scaling in water supplies, and problem of odour and corrosion in wastewater treatment due to its reduction to H2S.

A. Turbidimetric method

1. Principle

This method is used for the determination of sulphate ions. Sulphate ion (SO_4^{--}) is precipitated in an acetic acid medium with Barium chloride (BaCl2) so as to form Barium sulphate ($BaSO_4$) crystals of uniform size.

The reaction involved is given below:

$Ba^{++} + SO_4^{--} = BaSO_4$ (White suspension)

Light absorbance of the $BaSO_4$ suspension is measured by a photometer or the scattering of light by Nephelometer.

2. Apparatus and equipment

a. Magnetic stirrer

b. Colorimeter for use at 420mm or turbidimeter/nephelometer

c. Stopwatch

d. Nessler tubes, 100mL

e. Measuring spoon 0.2 to 0.3mL

3. Reagents and standards

a. Buffer solution A: dissolve 30g Magnesium chloride, MgCl2.6H2O, 5g Sodium acetate CH3COONa.3H2O, 1g Potassium nitrate, KNO3 and 20mL acetic acid, CH3COOH (99%) in 500mL distilled water and make up to 1000mL.

b. Buffer solution B: (required when the sample sulphate (SO4--) is less than 10mg/L). Dissolve 30g Magnesium chloride, MgCl2.6H2O, 5g sodium acetate, CH3COONa.3H2O, 1.0g of potassium nitrate, KNO3, 0.111 g of sodium sulphate, Na2SO4 and 20mL acetic acid (99%) in 500mL distilled water and make up to 1000mL.

c. Barium chloride: crystals, 20-30mesh.

d. Standard sulphate solution: dissolve 0.1479g anhydrous sodium sulphate, Na2SO4 in distilled water and dilute to 1000mL. 1L = 100 µg SO4--

4. Sample collection, preservation and storage

In presence of organic matter certain bacteria may reduce SO4-- to S--. To avoid this, store sample at 4°C.

5. Calibration

Prepare standard curve by with standard sulphate solution through entire procedure. Space standards at 5mg/L increment in the 0 to 40 mg/L range. Read mg SO4 -- present in the sample from the standard curve.

6. Procedure

a. Take suitable volume of sample and dilute to 100mL into a 250mL Erlenmeyer flask

b. Add 20mL buffer solution, mix well

c. Keep the flask constantly stirred with the help of stirrer. Add 1-spatula BaCl2 crystals with stirring. Continue stirring for 1 minute after addition of BaCl2

d. Pour suspension into an absorption cell of photometer and measure turbidity at 5±0.5 min

e. To correct for sample colour, and turbidity, run a blank to which BaCl2 is not added.

7. Calculation

mg SO4--/L = {mg (SO4--) x 1000} / {mL of sample}

Note: If buffer solution A was used, determine sulphate concentration directly from the calibration curve after subtracting sample absorbance before adding BaCl2. If buffer solution B was used, subtract sulphate concentration of blank from apparent sulphate concentration as determined above, because the calibration curve is not a straight line, this is not equivalent to subtracting blank

absorbance from sample absorbance.

8. Precision and Bias

Correct the colour and turbidity present in the original sample by running blanks from which the BaCl2 is not added. During preparation of standard curve, space the standards at 5mg/L increments in the 0 to 40mg/L sulphate range. Above 40mg/L the accuracy of the method decreases and the suspensions of barium sulphate lose stability. Check reliability of the calibration curve by running a

standard with every three or four unknown samples. Draw the fresh calibration curve with change of fresh stock of reagent or chemical (Barium chloride). The stirring time 1 min should be kept constant for samples and the standards.

Synthetic unknown sample containing 259mg/L sulphate, when analysed in different laboratories showed relative standard deviation of 9.1% and relative error 1.2%.

9. Interferences

Colour, turbidity and silica in the concentration of 500ppm interfere in this estimation. Filtration is adopted to remove colour and turbidity. In presence of organic matter, precipitation of barium sulphate may not be satisfactory.

17. IRON (Fe)

Introduction:

Iron occurs in the minerals as hematite, taconite and pyrite. It is widely used in steel and other alloys. Elevated iron levels in water can cause stains in plumbing, laundry and cooking utensils and can impart objectionable taste and colour to foods. The United Nations FAO recommended level for irrigation water is 5mg/L. The US EPA secondary drinking water standard MCL is 0.3mg/L. The BIS standard desirable limit is 0.3mg/L.

Phenanthroline method

Principle: Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1, 10-phenanthroline at pH 3.2 to 3.3. Three molecules of Phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The coloured solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of Phenanthroline. Colour standards are stable for at least 6 months.

Interference: Among the interfering substances are strong oxidising agents, cyanide, nitrite, and phosphates (polyphosphates more so than orthophosphate), chromium, zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5mg/L, and nickel in excess of 2mg/L.

Bismuth, cadmium, mercury, molybdate, and silver precipitate Phenanthroline. The initial boiling with acid converts polyphosphates to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidising reagents. In the presence of interfering metal ions, use a larger excess of Phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal ions are present, the extraction method may be used. If noticeable amounts of colour or organic matter are present, it may be necessary to evaporate the sample, gently ash residue,and re-dissolve in acid. Ashing may be carried out in silica, porcelain crucibles that have been boiled for several hours in 1+1 HCl. The presence of excessive amounts of organic matter may necessitate digestion before use of the extraction procedure.

Minimum detectable concentration: Dissolved or total concentrations of iron as low as 10µg/L can be determined with a spectrophotometer using cells with a 5 cm or longer light path. Carry a blank through the entire procedure to allow for correction.

2. Apparatus and equipment

i. Spectrophotometer: use a 510nm, providing a light path of 1cm or longer.

ii. Acid-washed glassware: wash all glassware with conc. hydrochloric acid (HCl) and rinse with distilled water before use to remove deposits of iron oxide.

iii. Separatory funnels: 125mL, with ground glass or TFE stopcocks and stoppers.

3. Reagents and standards

Use reagents and distilled water free from iron contamination

i. Hydrochloric acid, HCl, conc. containing less than 0.5 ppm iron.

ii. Hydroxylamine solution: Dissolve 10g NH2OH.HCl in 100mL water.

iii. Ammonium acetate buffer solution: Dissolve 250g NH4C2H3O2 in 150mL water. Add 70mL conc. (glacial) acetic acid. Because even a good grade of NH4C2H3O2 contains a significant amount of iron, prepare new reference standards with each buffer preparation.

iv. Sodium acetate solution: Dissolve 200g NaC2H3O2.3H2O in 800 mL water.

v. Phenanthroline solution: Dissolve 100mg 1, 10-phenanthroline monohydrate, C12H8N2.H2O, in 100mL water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops of conc. HCl are added to the water (note: One milliliter of this reagent is sufficient for no more than 100µg Fe).

vi. Stock iron solution: Use metal (1) or salt (2) for preparing the stock solution.

i. Use electrolytic iron wire, or "iron wire for standardizing" to prepare the solution. If necessary, clean the wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200mg wire and place in a 1000mL volumetric flask. Dissolve in 20mL 6N sulphuric acid (H2SO4) and dilute to mark with water; 1mL = 200µg Fe.

ii. If ferrous ammonium sulphate is preferred, slowly add 20mL conc.H2SO4 to 50mL water and dissolve 1.404g Fe (NH4)2 (SO4)2.6H2O.Add 0.02M potassium permanganate (KMnO4) drop-wise until a faint pink colour persists. Dilute to 1000mL with water and mix; 1mL = 200µg Fe.

Standard iron solutions: Prepare daily for use.

- Pipette 50mL stock solution into a 1000 mL volumetric flask and dilute to mark with water; 1mL = 10µg Fe.
- Pipette 5mL stock solution into a 1000 mL volumetric flask and dilute to mark with water; 1 mL =1µg Fe.

vii. Isopropyl ether.

Cautions: Ethers may form explosive peroxides; test before using.

28.7.3 Procedure

Total iron: Mix sample thoroughly and measure 50mL into a 125mL Erlenmeyer flask. If this sample volume contains more than 200 µg iron, use a smaller accurately measured portion and dilute to 50mL. Add 2mL conc. HCl and 1mL NH2OH.HCl solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until volume is reduced to 15 to 20mL. (If the sample is ashed, take up residue in 2mL conc. HCl and 5mL water). Cool to room temperature and transfer to a 50 or 100 mL volumetric flask or nessler tube. Add 10mL NH4C2H3O2 buffer solution and 4mL Phenanthroline solution and dilute to mark with water. Mix thoroughly and allow at least 10 to 15 min to maximum colour development.

Ferrous iron: Determine ferrous iron at sampling site because of the possibility of change in the ferrous-ferric ratio with time in acid solutions. To determine ferrous iron only, acidify a separate sample with 2mL conc. HCl/100mLsample at the time of collection. Fill bottle directly from sampling source and stopper. Immediately withdraw a 50mL portion of acidified sample and add 20mL Phenanthroline solution and 10mL NH4C2H3O2 solution with vigorous stirring. Dilute to 100mL and measure colour intensity within 5 to 10 min. Do not expose to sunlight. (Colour development is rapid in the presence of excess .Phenanthroline. The Phenanthroline volume given is suitable for less than 50µg total iron, if larger amounts are present, use a correspondingly larger volume of Phenanthroline or a more concentrated reagent. Excess Phenanthroline is required because of kinetics of the complexing process. Calculate ferric iron by subtracting ferrous from total iron.

Colour measurement: Prepare a series of standards by accurately pipetting calculated volumes of standard iron solutions (use weaker solution to measure 1 to 10µg portions) into 125mL Erlenmeyer flasks, diluting to 50mL by adding measured volumes of water.

For photometric measurement, use light path at 510nm. Read standards against distilled water set at zero absorbance and plot a calibration curve, including a blank.If samples are coloured or turbid, carry a second set of samples through all steps of the procedure without adding Phenanthroline.

Samples containing organic interferences:

Digest samples containing substantial amounts of organic substances.

a. From the digested sample, pipette 10.0 mL or other suitable portion containing 20 to 500µg Fe into a 125mL separatory funnel. If the volume taken is less than 10mL, add distilled water to make up to 10mL. To the separatory funnel add 15 mL conc. HCl for a 10mL aqueous volume; or, if the portion taken was greater than 10mL. Add 1.5mL conc.HCl/mL of sample. Mix, cool to roomtemperature.

b. Add 1mL NH2OH.HCl solution, 10mL Phenanthroline solution and 10mL NaC2H3O2 solution. Dilute to 100mL with water, mix thoroughly and let stand for 10 min. Measure absorbance at 510 nm using a 5cm absorption cell for amounts of iron less than 100 µg or 1cm cell for quantities from 100 to 500µg. As reference, use either distilled water or a sample blank prepared by carrying the specified quantities of acids through the entire analytical procedure. If distilled water is used as reference, correct sample absorbance by subtracting absorbance of a sample blank. Determine micrograms of iron in the samplefrom the absorbance (corrected, if necessary) by reference to the calibration curve prepared by using a suitable range of iron standards containing the same amounts of Phenanthroline, hydroxylamine and sodium acetate as the sample.

28.7.4 Calculation

mg Fe/L = µg Fe (in 100mL final volume) / mL sample

or for digested samples

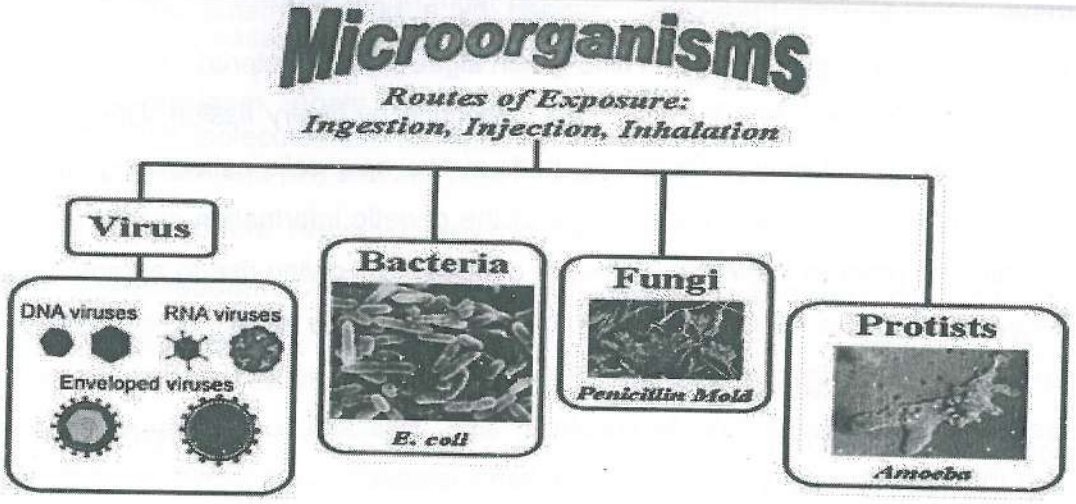
mg Fe/L. = {µg Fe (in 100mL final volume) / mL sample} x {100 / mL portion}

Report details of sample collection, storage and pre-treatment, if they are pertinent to interpretation of results.

MICROBIOLOGY

What is microbiology **:-**

Microbiology is the scientific **study** of Microorganisms , those organisms are Extremely small to see with the naked eye and include organisms like bacteria, algae, fungi, protozoa and viruses. Those Microorganisms having diameter of 1 mm (or) less.



Microbiology based on study subdivided into divisions including bacteriology, virology, mycology, parasitology and others.

- These micro organisms are unknown till the invention of microscope in 17th century by the scientist " **Antonie van Leeuwenhoek** " due to his inventions he called as "father of Microbiology"

In now a days many microscopes are invented for study of microorganisms like compound , Dark field, fluorescent, phasecontrast, Electron microscope, ultrasonic microscope etc...

What is Bacteria?

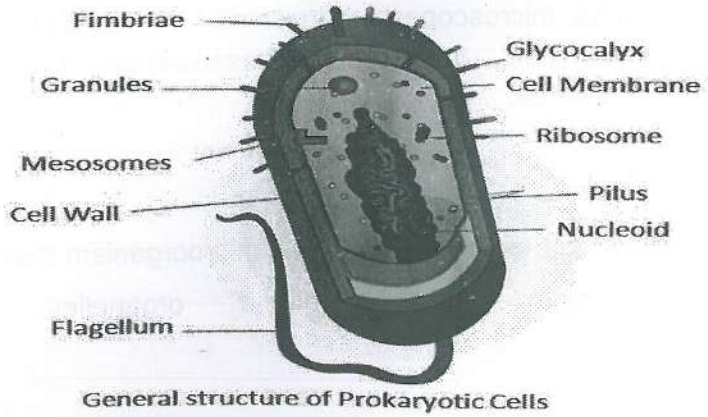
A bacterium is a single-celled (**unicellular**) microorganism that does not have a nucleus or any other membrane-bound organelles. Bacteria are

'prokaryotes.'organisms In Greek, 'prokaryote' literally means 'before the nut' (where 'the nut' is the nucleus.)

Bacteria are among the simplest, smallest, and most abundant organisms on earth. Most bacteria are only 1 micrometer (μm) in diameter, but they can range in size from 0.1 μm to greater than 10 μm. Bacteria are *procaryotic*organisms; that is, they do not have an organized nucleus surrounded by a nuclear membrane. Procaryotic microorganisms include bacteria and blue-green algae (cyanobacteria). Bacteria contain a single strand of DNA, and they typically reproduce by *binary fission*. During binary fission, a single cell divides transversely to form two new cells called *daughter cells*. Each daughter cell contains an exact copy of the genetic information contained in the parent cell. The process continues with each daughter cell giving rise to a generation of two new cells. The way that bacteria populations increase is an example of *geometric progression*: 1 cell 2 cells 4 cells 8 cells 16 cells, etc. The *generation time* is the time required for a given population to double in size. This time can be as short as 20 minutes for some bacteria species (*e.g.*, *Escherichia coli*).

All bacteria are *unicellular* (single-celled) organisms. Two methods by which bacteria can be grouped are by *cell shape* and by *differential stains*. There are three key shapes: bacilli (rods), cocci (spherical or spheroid), and spirilla (spiral or corkscrew). Cells can occur either individually or as groups of cells. Grouped cells neither communicate nor cooperate with each other; however, the configurations that are observed for a particular species are fairly constant. Arrangements of spherical cells can be used in taxonomy: diplococci (paired cells), streptococci (cell chains), tetracocci (four cells arranged in a square), and staphylococci (grape-like clusters).

Bacteria adapt to become well-suited to their environments, and therefore come in many shapes and forms. However, they all have a few parts in common.



General structure of Prokaryotic Cells

1. Capsule: A protective, often slimy, coating, often of sugars, that helps to protect the bacterium. It also makes bacteria **virulent**. This means the bacteria is more likely to cause disease, since it aids the cell in survival against attack. For example, the bacteria may survive an attack from the human body's immune system.

2. Cell wall: In bacteria, the cell wall is usually made of **peptidoglycan**, a protein and sugar compound. This structure gives the cell some rigidity and protection.

3. Cell membrane: As in most cells, the bacterium's plasma membrane acts by coordinating the passage of molecules into and out of the cell.

4. **Cytoplasm**: Again, as in many cells, the cytoplasm serves as a medium through which molecules are transported, as well as a system to maintain conditions (like temperature and pH) that are best for the cell.

5. **Ribosomes**:.Are machinery for protein synthesis.

6. **Nucleosome**: A basic unit of chromatin, which won't be covered in this lesson.

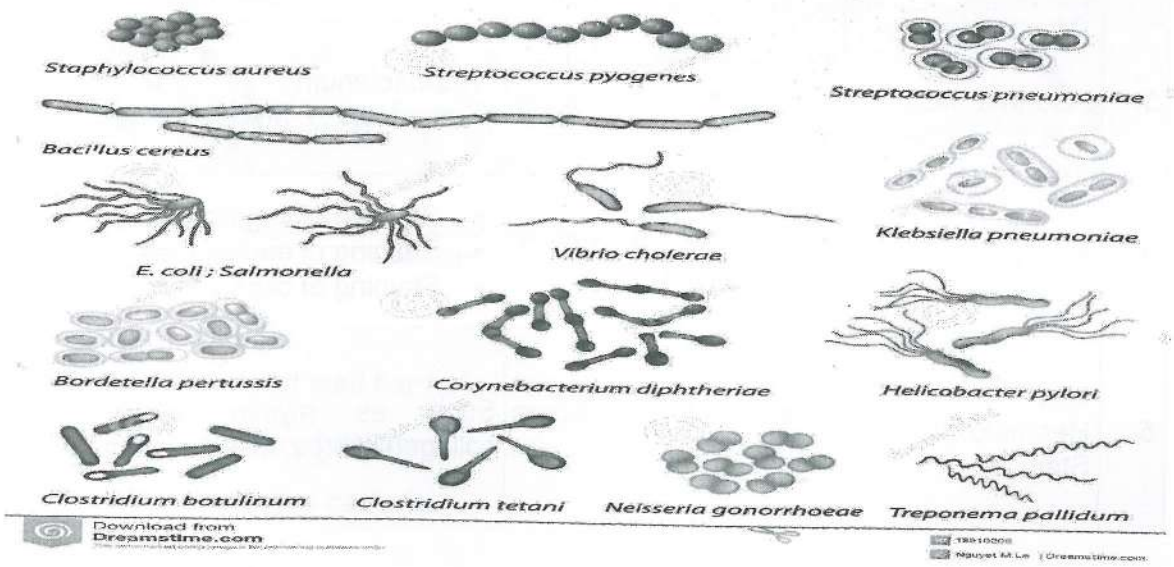
7. **Nucleoid**: This is the region where the bacterium's DNA is located. Again, it's not the same as a nucleus because it's not surrounded by a membrane.

8. **Flagellum**: In many bacteria, a flagellum is present, and it is the locomotory organelle, means by which the cell moves around.

Shape of bacteria :

Bacteria are classified into 5 groups according to their basic shapes: **spherical** (cocci), rod (bacilli), **spiral**(spirilla), comma (vibrios) or corkscrew (spirochaetes). They can exist as single cells, in pairs, chains or clusters.

Shapes of Bacteria



What is staining:-

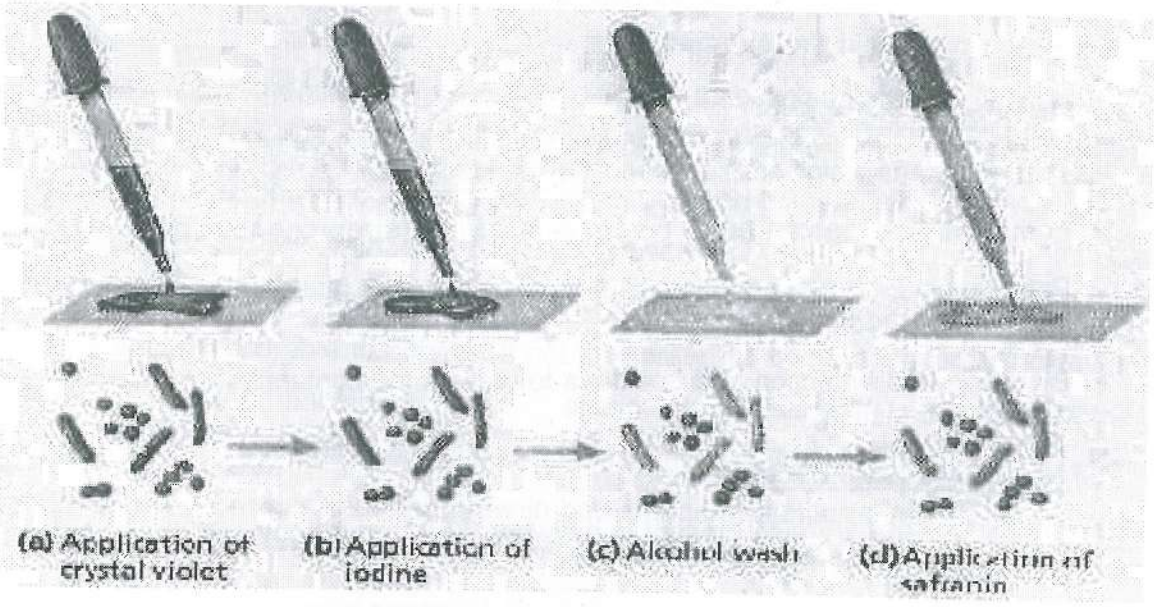
Staining simply means coloring of the micro organisms with the dye that clear and explain different important structures of microorganisms including bacteria, virus, protozoa and etc.

Importance of Staining:-

- In microbiology the concept of *staining* is very *important* because it highlights the structures of microorganisms allowing them to be seen under a microscope (simple and electron microscope).
- It is also used to differentiate different microorganisms.
- Used for the identification of microorganisms like bacteria which may be either **gram positive or gram negative**.
-

Sr. No.	Type of Staining	Purpose of Staining	Examples/Chemicals
1-	Simple Stain	To highlight the total count of bacteria	▪ Methylene blue ▪ Crystal violet
2-	Gram Staining	Gram negative bacteria give pink colour Gram positive bacteria give purple colour	▪ Saffranine ▪ Crystal violet
3-	Acid-fast Stain	Tc identify the bacteria having cell wall of waxy material	Mycobacterium tuberculosis / Mycobacteriumleprae
4-	Special Stain	To isolate the specific part of bacteria	▪ Staining of flagella ▪ Staining of endospores ▪ Staining of capsule
5-	Iron-Heamatoxylin Stain	It is used to stain tissue components	Such as myelin, elastic and collagenicfibres, muscle striations etc

6-	Wheatley Trachoma Stain	Easiest &quickest method to stain protozoa	Wheatley's stain for intestinal amebasandllagellates
7-	Positive staining of Viruses on Grid	To identify the viruses under electron microscope	Uranyl Acetate
8-	Negative Staining Of Viruses	To identify the viruses under electron microscope	Uranyl Acetate
9-	Gomori Methena mine Silver (GMS) Staining	to look for fungi in tissues and incytology specimens	GMS staining of *Aspergillus.*
10-	Periodic acid-Schiff(PAS) Staining	to look for fungi in tissues and incytology specimens	PAS staining of *Cryptococcus*



Based on their cell wall bacteria are divided into 2 types

1) Gram Positive bacteria
2) Gram negative bacteria.

Gram-positive bacteria are bacteria that give a positive result in the Gram stain test, which is traditionally used to quickly classify bacteria into two broad categories according to their cell wall.

Gram-positive bacteria take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through a microscope. This is because the thick peptidoglycan layer in the bacterial cell wallretains the stain after it is washed away from the rest of the sample, in the decolorization stage of the test.

Gram-negative bacteria are bacteria that do not retain the crystal violet dye in the Gram stain protocol. Gram-negative bacteria imparts secondary stain Saffranine , so that , it appear red or pink following a Gram stain procedure due to the effects of the counterstain (for example saffranine)

Classification of Bacteria based onharmful & not harmful criteria:

Based on which bacteria can harmful to us (or) not harmful to us .bacteria are divided into 2 types

A) Pathogenic Bacteria

B) Non Pathogenic Bacteria.

A) Pathogenic Bacteria :-

- These bacteria which are capable of causing disease when enters in to body.
- Pneumonia can be caused by the bacteria such as**(streptococcus pneumonia)**
- Pathogenic Bacteria are also cause of high infant mortality rates in developing countries like leprosy disease.
- By usage of disinfection to kill bacteria to prevent the contamination & further reduce the risk of infection.

B) Non Pathogenic Bacteria :-

- These bacteria are also present in anywhere (or) in our body and do not cause any disease or harmful to us.
- For Examplecertain strains ofE.Coli strains normally found in the gastro intestinal tract , have ability to stimulate the immune response in humans.
- Non pathogenic bacteria may inhibit the growth & reproduction of harmful bacteria.
- Lactobacillus acidophilus bacteria in a part of normal intestinal flora helps in the intestine.
- Bacteroidsprevents Pathogenic Bacteria from colonising intestines.

Classification: Based on Oxygen Requirement

- ***Obligate* (or *strict*) *aerobes***:
- The presence of oxygen is required (*e.g.*, *Pseudomonas fluorescens*)
- ***Obligate* (or *strict*) *anaerobes***:
- The absence of oxygen is required, oxygen is toxic to the cells (*e.g.*, *Clostridium botulinum, C. tetani*)
- ***Facultative anaerobes***:
- They can survive with or without oxygen (*e.g.*, *Escherichia coli*)
- ***Microaerophiles***: They require low concentrations of oxygen and don't do well either at atmospheric oxygen concentrations or without oxygen (*e.g.*, *Sphaerotilusnatans, Enterobacteraerogenes*)
- Bacteria cannot control their own temperature. The range of temperatures that they can withstand categorizes bacteria as either ***stenothermal*** or ***eurythermal***:
- ***Stenothermal***: They can only survive over a very narrow temperature range (< 10 ^{0}C range)
- ***Eurythermal***: They can survive over a wide temperature range
- The optimum temperature for growth is the temperature at which the fastest growth rate occurs. Bacteria are classified as *psychrophiles*, *mesophiles*, or *thermophiles* based on their optimum growth temperature:
- ***Psychrophiles***: optimum temperature occurs between 0 and 20 C
- ***Mesophiles***: optimum temperature occurs between 20 and 45 C
- ***Thermophiles***: optimum temperature occurs between 45 and 60 C
- Many bacteria are able to survive, if not proliferate, at low temperatures; however, only some bacteria are able to survive at elevated temperatures. *Thermusaquaticus* and *Bacillus stearothermosphilus* are non-sporeforming bacteria that can grow in hot springs at temperatures above 70 and 55^{0}C, respectively. Some bacteria are able to survive high temperatures because they form spores. *Spores* are special cells that are resistant to harsh environmental conditions. Once conditions become favourable, the cells return to the *vegetative* (or actively growing) state.
- Bacteria can be useful to humans in many ways. Bacteria decompose many types of organic substances and are currently being investigated as a means of

decomposing unwanted synthetic chemicals (*e.g.*, pesticides, dyes, and petroleum) that are released into the environment.

1. Bacteria and water:

Water borne pathogens can enter the human body through intact or compromised skin, inhalation, ingestion, aspiration, or by direct contact with mucosa of the eye, ear, nose, mouth and genitals, and cause disease. Besides failure of potable water systems, which usually contribute to the greatest number of outbreaks of waterborne diseases, common outdoor recreational activities such as swimming, boating, bathing, camping and hiking, all place humans at risk of waterborne diseases from ingestion or direct contact with contaminated water. A risk analysis approach is required for overcoming the problems with waterborne diseases and water quality monitoring. While this could be achieved relatively easily with respect to chemical contaminants, and standards could be set up forchemical contaminants, setting standards for microbial contaminants has proved more difficult, because, (a) methods for detection of many pathogens are not yet available; (b) days to weeks are some times required to obtain results; and (c) expensive and time consuming methods are often required to detect and quantify the presence of specific pathogens.

To overcome these difficulties, the concept of testing water samples for indicator organisms was introduced as far back as the turn of the 19th century. The concept was developed for assessing faecal contamination of water and the concept relies on the fact that certain non-pathogenic bacteria present in water can be easily isolated and quantified by simple bacteriological methods; detection of these bacteria would essentially mean that faecal contamination has occurred and also suggest that enteric pathogens also may be present. The essential criteria for indicator organisms are given in the box below:

Criteria for indicator micro-organisms in water:

- Should be useful for all types of water
- Should be present whenever enteric pathogens are present
- Should have a reasonable longer survival time than the hardest enteric pathogen
- Should not grow in water
- Testing method for the organism should be easy to perform

- ❖ Density of the indicator should have some direct relationship to the degree of fecal pollution
- ❖ Should be a member of the intestinal micro-flora of humans and warm-blooded animals

It should, however, be appreciated that the choice of monitoring indicator(s) presupposes an understanding of the parameters to be measured and the relationship of the indicator(s) to the pathogen(s). No single indicator provides assurance that water is pathogen-free. Pathogens such as *Psedomonas, Aeromonas, Plesidomnas, Yersinia, Vibrio, Legionella and Mycobacterium* may not correlate with the most commonly used coliform indicators, and also, traditional bacteria indicators may not also correlate with viruses and parasites in pristine waters or ground waters. A brief consideration of the common indicator organisms that are currently employed in qualitative/quantitative tests for water would be appropriate here.

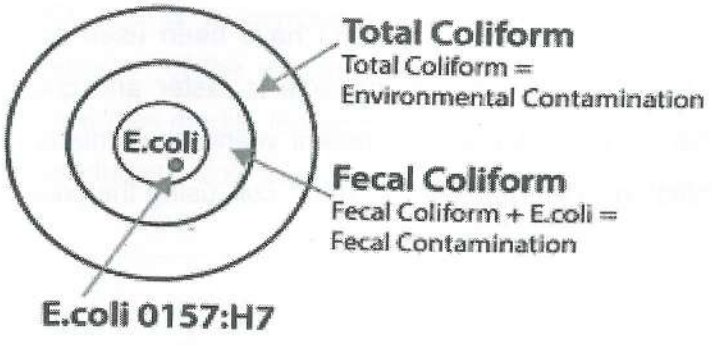
2. Indicator microorganisms of faecalpollution:

1. Coliform organisms (total coliforms):

Coliform bacteria are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria.

- ➢ **Coliform bacteria** ferment lactose with the production of acid and gas when incubated at 35–37°C.
- ➢ Coliforms can be found in the aquatic environment, in soil and on vegetation
- ➢ they are universally present in large numbers in the faeces of warm-blooded animals.
- ➢ . While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present.
- ➢ Coliform procedures are performed in aerobic respiration or anaerobic conditions.
- ➢ Such pathogens include disease-causing bacteria, viruses, or protozoa Example – **E-coli.**

Total Coliform, Fecal Coliform and E.coli



Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. The term "coliform organisms" refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35–37°C with the production of acid, gas, and aldehyde within 24–48 hours. They are also oxidase-negative and nonspore- forming and display b-galactosidase activity. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*. However, as defined by modern taxonomical methods, the group is heterogeneous. It includes lactosefermenting bacteria, such as *Enterobacter cloacae* and *Citrobacterfreundii*, which can be found in both faeces and the environment (nutrient-rich waters, soil, decaying plant material) as well as in drinking-water containing relatively high concentrations of nutrients, as well as species that are rarely, if ever, found in faeces and may multiply in relatively good-quality drinking-water, e.g. *Serratiafonticola*, *Rabnellaaquatilis*, and *Buttiauxellaagrestis*. The existence both of non-faecal bacteria that fit the definitions of coliform bacteria and of lactose-negative coliform bacteria limits the applicability of this group as an indicator of faecal pollution. Coliform bacteria should not be detectable in treated water supplies and, if found, suggest inadequate treatment, post treatment contamination, or excessive nutrients. The coliform test can therefore be used as an indicator both of treatment efficiency and of the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in drinking water, the coliform test is still useful for monitoring the microbial quality of treated piped water supplies. If there is any doubt, especially when coliform organisms are found in the absence of thermotolerant coliforms and *E. coli*, identification to the species level or analyses for other indicator organisms may be undertaken to investigate the nature of the contamination.

Coliform bacteria include all aerobic and facultative anaerobic gram negative, non-spore forming, rod shaped bacteria when incubated at 35^0C, can ferment lactose and produce gas (CO2) within 48 hrs. Coliform bacteria have been used to evaluate the general quality of water. Testing for coliform bacteria is faster and cheaper than testing for specific organisms and pathogens. In recent years, new criteria have been added to traditional definition of coliform bacteria and E. *coli*, using the presence of characteristic

enzymatic activities. Definition of coliform or faecal coliform basically relies on the activity of a single enzyme β- *galactosidase*. The new enzymatic definition of total coliform bacteria is based on the presence of β- *galactosidase;* and that of E. *coli* is based on the enzymatic action of β- *glucuronidase*. This aspect is well utilized in developing very sensitive and specific presence-absence (P-A) tests for detection of total coliforms and E *coli*.

Deficiencies with the use of coliform bacteria as indicator of water quality include:

- Re-growth in aquatic environments
- Re-growth in distribution systems, including biofilm colonization
- Suppression by high background bacterial growth
- Not indicative of health threat
- No relationship with enteric protozoan and viral concentration.

Coliform bacteria had been used historically to assess the microbial quality of drinking water. However, by the 1980s it had become quite clear that coliform bacteria did not indicate the presence of pathogenic waterborne *Giardia* or enteric viruses. Numerous outbreaks had occurred. In whichcoliform standards were met, because of greater resistance of viruses and *Giardia* to chlorination. A new approach was needed to ensure the microbial safety of drinking water. Most Probable Number (MPN), the Membrane filter (MF) and presence-absence (P/A) tests are used to detect the total coliforms.

1. Faecal coliform bacteria:

Fecal coliforms are the coliform bacteria that originate specifically from the intestinal tract of warm-blooded animals (*e.g.*, humans, beavers, racoons, etc.). They are cultured by increasing the incubation temperature to 44.5ºC and using somewhat different growth media.

Faecal coliform bacteria are differentiated in the laboratory by their ability to ferment lactose, with production of acid and gas at 44.5ºC within 24 h. Faecal coliforms pose some of the same limitations as those posed by coliforms (Regrowth in distribution system, less resistance to water treatment than viruses and protozoa, etc.) Faecal coliforms are also detected by similar methods (MPN, MF and P/A) used for total coliforms. ***Escherichia coli*** is a member of the family Enterobacteriaceae, and is characterized by possession of the enzymes b-galactosidase and b-glucuronidase. It grows at 44–45°C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. However, some strains can grow

at 37 °C but not at 44–45°C, and some do not produce gas. *E. coli* does not produce oxidase or hydrolyseurea.Complete identification of the organism is too complicated for routine use, but a number of tests have been developed for rapid and reliable identification. Some of these methods have been standardized at international and national levels and accepted for routine use; others are still being developed or evaluated.

What is Ecoli :-

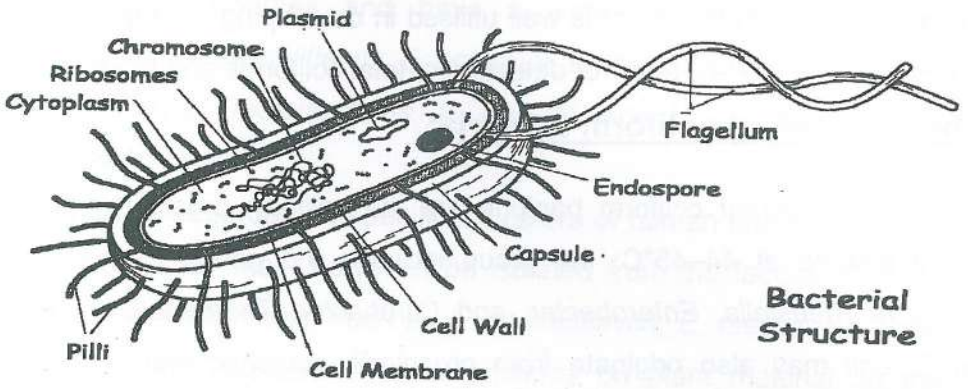
Ecoli means *Escherichia coli*. Escherichia coli (*E. coli*) bacteria normally live in the intestines of people and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons.

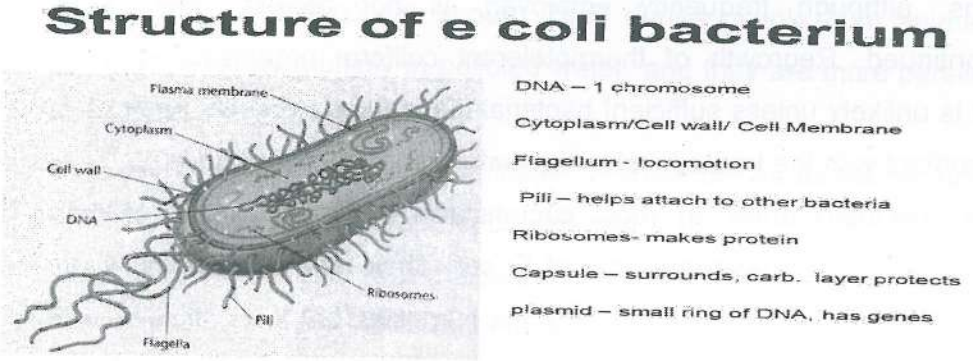
- E coli is a rod-shaped member of the coliforms group.
- It can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliform test.
- By its growth and color reaction on certain types of culture media. When cultured on an **eosin methylene blue (EMB)** plate, a positive result for *E. coli* is metallic green colonies on a dark purple media.
- Escherichia coli have an incubation period of 12–72 hours with the optimal growth temperature being 30–37°C.
- Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination.
- Most strains of *E. coli* are harmless, but some can cause serious illness in humans. Infection symptoms and signs includes bloody diarrhea,stomach cramps, vomiting and fever..etc.
- The bacteria can also cause pneumonia, other respiratory iillnesses and urinary tract infections.

Various pathogenic Ecoli strains:-

- Enterotoxigenic *E. coli* (ETEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroinvasive *E. coli* (EIEC)

- Enterohemorrhagic *E. coli* (EHEC)
- Uropathogenic *E. coli* (UPEC)
- Verotoxin-producing *E. coli*
- *E. coli* O157:H7 is an enterohemorrhagic strain also 2006 North American *E. coli* outbreak
- *E. coli* O104:H4, also 2011 *E. coli* O104:H4 outbreak
- *Escherichia coli* O121
- *Escherichia coli* O104:H21
- *Escherichia coli* K1, meningitis
- *Escherichia coli* NC101





Escherichia coli is abundant in human and animal faeces; in fresh faeces it may attain concentrations of 109 per gram. It is found in sewage, untreated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, wild animals, or agricultural activity. Recently, it has been Suggested that *E. coli* may be

present or even multiply in tropical waters not subject to human faecal pollution. However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded. Because animals can transmit pathogens that are infective in humans, the presence of *E. coli* or thermotolerant coliform bacteria must not be ignored, because the presumption remains that the water has been faecally contaminated and that treatment has been ineffective.

In recent years, new criteria have been added to traditional definition of coliform bacteria and *E. coli,* using the presence of characteristic enzymatic activities. Definition of coliform or faecal coliform basically relies on the activity of a single enzyme b-*galactosidase*. The new enzymatic definition of TOTAL COLIFORM bacteria is based on the presence of β- *galactosidase*; and that of *E. coli* is based on the enzymatic action of β - *glucuronidase*. This aspect is well utilised in developing very sensitive and specific presence-absence (P-A) tests for detection of total coliforms and *E. coli.*

3.Thermotolerant coliform bacteria:

Thermotolerant coliform bacteria are the coliform organisms that are able to ferment lactose at 44–45°C; the group includes the genus *Escherichia* and some species of *Klebsiella*, *Enterobacter*, and *Citrobacter*. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. For this reason, the term "faecal" coliforms, although frequently employed, is not correct, and its use should bediscontinued. Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present, unsuitable materials are in contact with the treated water, the water temperature is above 13 °C, and there is no free residualchlorine. In most circumstances, concentrations of thermotolerant coliforms are directly related to that of *E. coli*. Their use in assessing water quality is therefore considered acceptable for routine purposes, but the limitations with regard to specificity should always beborne in mind when the data are interpreted. If high counts of thermotolerant coliforms are found in the absence of detectable sanitary hazards, additional confirmatory tests specific for *E. coli* should be carried out. National reference laboratories developing national standard methods are advised to examine the specificity of the thermotolerant coliform test for *E. coli*under local conditions. Because thermotolerant coliform organisms are readily detected, they have an important secondary role as indicators of the efficiency of water-treatment processesIn removing

faecal bacteria. They may therefore be used in assessing the degree of treatment necessary for waters of different quality and for defining performance targets for removal of bacteria.

Two other groups of bacteria that are present in faeces are: fecal streptococci and *Clostridium*

a. Faecal streptococci:

Faecal streptococci are those streptococci generally present in the faeces of humans and animals. All possess the Lancefield group D antigen. Taxonomically, they belong to the genera *Enterococcus* and *Streptococcus*. The taxonomy of Enterococci has recently undergone important changes, and detailed knowledge of the ecology of many of the new species is lacking; the genus *Enterococcus* now includes all streptococci that share certain biochemical properties and have a wide tolerance of adverse growth conditions—*E.avium*, *E. casseliflavus*, *E. cecorum*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodouratus*, *E. mundtii*, and *E. solitarius*. Most of these species are of faecal origin

and can generally be regarded as specific indicators of human faecal pollution for most practical purposes. They may, however, be isolated from the faeces of animals, and certain species and subspecies, such as *E. casseliflavus*, *E. faecalis*var. *liquefaciens*, *E.malodouratus*, and *E. solitarius*, occur primarily on plant material. In the genus *Streptococcus*, only *S. bovis*and*S. equinus*possess the group D antigen and therefore belong to the faecal streptococcus group. They derive mainly from animal faeces. Faecal streptococci rarely multiply in polluted water, and they are more persistent than *E. coli* and coliform bacteria.

Their primary value in water-quality examination is therefore as additional indicators of treatment efficiency. Moreover, streptococci are highly resistant to drying and may be valuable for routine control after new mains are laid or distribution systems are repaired, or for detecting pollution of ground waters or surface waters by surface run-off.

Faecal streptococci include the gram-positive, Lancefield Group-D streptococci, belonging to the genera *Enterococcus* and *Streptococcus.*

The genus *Enterococcus* is differentiated from other streptococci by ability to grow in 6.5% NaCl, pH 9.6 and 45°C (includes spp. E. *avivum,* E. *faecium,* E. *faecalis*and E. *gallinarium).* E. faecium and E. faecalis are more specific to human gut. In the genus

Streptococcus, only S. *bovis*and S. *equines* are considered to be true faecal streptococci have certain advantages over the coliforms, as indicators:

They rarely multiply in water

- More resistant to environmental stress and chlorinated than coliforms
- Persist longer in the Environment
- Suggested as useful indicators for recreational waters

Both MPN and MF methods may be used for isolation and enumeration of Faecal streptococci. .Fecal streptococci and Enterococci are terms that have been used interchangeably; however, there are some differences between the two groups.

Faecal streptococci

Entercoccus Group	|-- *S.faecalis*	-- |
	| *S.faecalis* subsp. *liquefaciens*	|
	| *S.faecalis* subsp. *zymogenes*	|
	|-- *S.faecium*	|
	S.bovis	|
	S.equinus	--|
	S.avium	

Fecal streptococci indicate the presence of fecal contamination by warm-blooded animals. Unlike coliforms, fecal streptococcal bacteria are not known to multiply in the environment. Also, they tend to die-off more quickly than coliforms.

The ratio of fecal coliforms to fecal streptococci (FC/FS) can provide information on the source of contamination;

FC/FS ratios

Source	Ratio
Man	4.4
Duck	0.6
Sheep	0.4
Chicken	0.4
Pig	0.4
Cow	0.2

Turkey	0.1

However, several precautions are in order when using these ratios:

(1) Bacterial concentrations can be greatly variable if the pH is outside of the 4.0 to 9.0 range,

(2) The faster die-off rate of fecal streptococci will alter the ratio as time from contamination increases,

(3) Pollution from several sources can alter the ratio and confuse the issue,

(4) FC/FS ratios have been of limited value in identifying pollution sources in irrigation returns, bays, estuaries, and marine waters, and

(5) Ratios should not be used when fecal streptococcal counts are less than 100/100 mL.

4. Bacterial Diseases:

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Botulism	*Clostridium botulinum*	Bacteria can enter through wound from contaminated water sources. Can enter the gastrointestinal tract through consumption of contaminated drinking water	Dry mouth, blurred or double vision, difficulty swallowing, muscle weakness, difficulty breathing, vomiting and sometimes diarrhea Death is usually caused by respiratory failure.
Campylobacteriosis	Most commonly caused by *Campylobacter jejuni*	Drinking water contaminated with feces	Produces dysentery-like symptoms along with a high fever . Usually lasts 2–10 days.

Cholera	Spread by the bacterium *Vibrio cholerae*	Drinking water contaminated with the bacterium	It causes fatal illnesses. Symptoms include watery diarrhea, nausea, cramps, nosebleed, rapid pulse, vomiting, and hypovolemic shock (in severe cases), at which point death can occur in 12–18 hours.
E. coli Infection	Certain strains of *Escherichia coli* (commonly *E. coli*)	Water contaminated with the bacteria	Mostly diarrhea. Can cause death in immunocompromised individuals , the very young, and the elderly due to dehydration from prolonged illness.
M. marinum infection	*Mycobacterium marinum*	Naturally occurs in water, in swimming pools or more frequently aquariums ; it mostly infects immunocompromisedindividuals	Symptoms include lesions typically located on the elbows, knees, and feet painless or painful.
Dysentery	Caused by a number of species in the genera *Shigella* and *Salmonella*with the most common being *Shigelladysenteriae*	Water contaminated with the bacterium	Frequent passage of feces with blood or mucus and in some cases vomiting of blood.
Legionellosis (two distinct forms: Legionnaires' disease and Pontiac fever)	Caused by bacteria belonging to genus *Legionella* (90% of cases caused by *Legionella pneumophila*)	Legionella is a very common organism that reproduces to high numbers in warm water; but only causes severe disease when aerosolized.	Pontiac fever produces milder symptoms resembling acute influenza without pneumonia Legionnaires' disease has severe symptoms such as fever, chills, pneumonia ataxia, anorexia, muscle aches, and occasionally diarrhea and vomiting
Leptospirosis	Caused by bacterium of genus *Leptospira*	Water contaminated by the animal urine carrying the bacteria	Begins with flu-like symptoms The second phase occurs meningitis, liver damage (causes jaundice), and renal failure
Otitis Externa(swimmer's ear)	Caused by a number of bacterial and fungalspecies.	Swimming in water contaminated by the responsible pathogens	Ear canal swells, causing pain and tenderness to the touch
Salmonell	Caused by many	Drinking water	Symptoms include diarrhea, fever,

osis	bacteria of genus *Salmonella*	contaminated with the bacteria. More common as a food borne illness.	vomiting, and abdominal cramps
Typhoid fever	*Salmonella typhi*	Ingestion of water contaminated with fecesof an infected person	Characterized by sustained fever up to 40 °C (104 °F), profuse sweating; diarrhea may occur. Symptoms progress to delirium, and the spleen and liver enlarge if untreated. In this case it can last up to four weeks and cause death. Some people with typhoid fever develop a rash called "rose spots", small red spots on the abdomen and chest.
Vibrio Illness	*Vibrio vulnificus*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus*	Can enter wounds from contaminated water. Also acquired by drinking contaminated water or eating undercooked oysters .	Symptoms include abdominal tenderness, agitation, bloody stools, chills, confusion, difficulty paying attention (attention deficit), delirium, fluctuating mood, hallucination, nosebleeds, severe fatigue, slow, sluggish, lethargic feeling, weakness.

5.VIRUSES

WHAT IS VIRUS :

A **virus** is a small infectious agent that replicates only inside the living cells of other organisms. The study of viruses is known as virology. viruses exist in the form of independent particles. These **viral particles**, also known as **virions**, Viruses spread in many ways; viruses in plants are often transmitted from plant to plant by insects that feed on plant sap, such as aphids; viruses in animals can be carried by blood-sucking insects. These disease-bearing organisms are known as vectors

- Viruses are major cause of human water born diseases
- Pathogenic Viruses those are responsible for gastroenteritis, hepatitis.
- Some virus can be hosting specific HIV& some can be less host specific viruses like influenza.
- Adenovirus &heeriridae virus can cause water born diseases

Properties of virus

Virus ranges(20-300) nm (Parvov. (20nm) Pox v. (400nm)

Viruses are spherical, rectangular, bullet & pleomorphic

Virusexhibit Helical symmetry & icosahedral symmetry.
Viral genome contains either DNA or RNA
Capsid is outerlayer of virusphospholipid bilayer acts as envelope.
Some virus possess their own enzymes(reverse transcriptase)
Most viruses are heat labile.Virusesare inactivated 60°C for 30 minutes or 100°C for few seconds.
Virus can be stored at -40°C to -70°C(lyophilization or freeze drying.)
Both non-ionizing and ionizing radiation can kill virus.
UV rays forms dimer, X-rays causes break of genome)
Chloroform, ether & bile salt destroy viruses (lipid solubiliation.)
Most viruses are destroyed by oxidizing agents(H_2O_2, iodine etc)
Many viruses are resistant to phenol and chlorination

Structure:-

Viruses display a wide diversity of shapes and sizes, called *morphologies*. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and300 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with an optical microscope .

Shapes of viruses

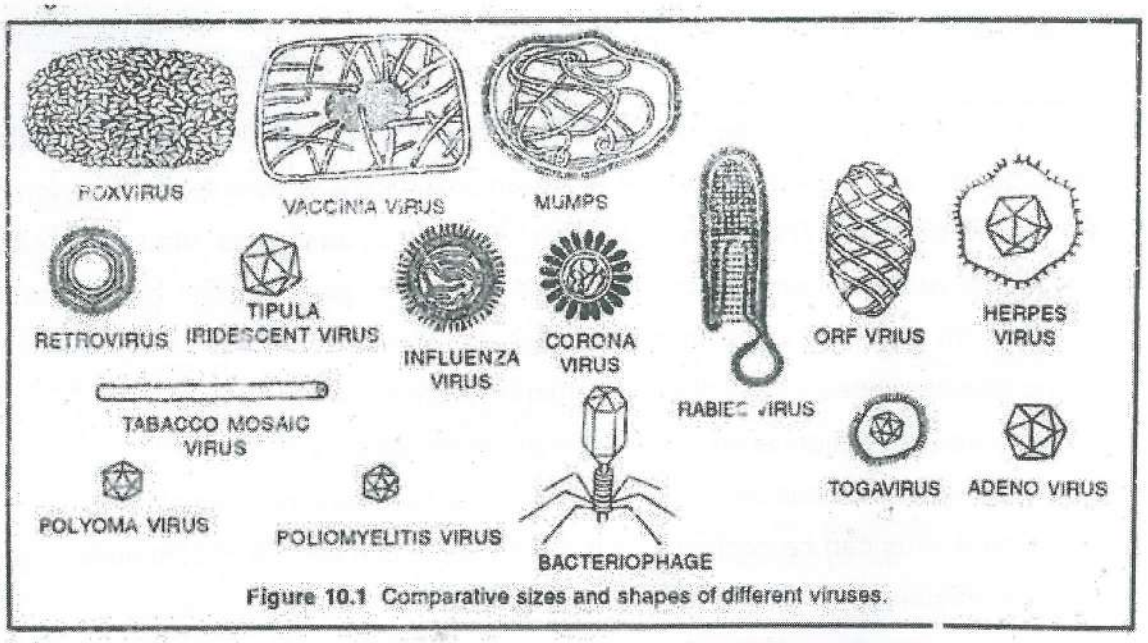


Figure 10.1 Comparative sizes and shapes of different viruses.

Viral diseases

Disease and Transmission	Viral Agent	Sources of Agent in Water Supply	General Symptoms
SARS (Severe Acute Respiratory Syndrome)	Coronavirus	Manifests itself in improperly treated water	Symptoms include fever, myalgia, lethargy, gastrointestinal symptoms, cough, and sore throat
Hepatitis A	Hepatitis A virus (HAV)	Can manifest itself in water (and food)	Symptoms are only acute (no chronic stage to the virus) and include Fatigue, fever, abdominal pain, nausea, diarrhea, weight loss, itching, jaundice and depression.
Poliomyelitis(Polio)	Poliovirus	Enters water through the feces of infected individuals	90-95% of patients show no symptoms, 4-8% have minor symptoms (comparatively) with delirium, headache, fever, and occasional seizures, and spastic paralysis, 1% have symptoms of non-paralytic aseptic meningitis. The rest have serious symptoms resulting in paralysis or death
Polyomavirus infection	Two of Polyomavirus: JC virus and BK virus	Very widespread, can manifest itself in water, ~80% of the population	BK virus produces a mild respiratory infection and can infect the kidneys of immunosuppressed transplantpatients. JC

		has antibodies to Polyomavirus	virus infects the respiratory system, kidneys or can cause progressive multifocal leukoencephalopathy in the brain (which is fatal).

6.FUNGI

What is fungi :-

Fungus, plural **fungi**, any of about 99,000 known species of organisms of the kingdom **Fungi**, which includes the yeasts, rusts, smuts, mildews, molds, and mushrooms. ... Many **fungi** are free-living in soil or water; others form parasitic or symbiotic relationships with plants or animals.

A **fungus** is any member of the group of eukaryotic organisms that includes microorganisms These organisms are classified as a kingdom, **Fungi**, which is separate from the other eukaryotic life kingdoms of plants and animals

General properties of fungi:

Fungi are Eukaryotic posses cell organelles including nucleus, mitochondria, golgi apparatus, E.R &vacuoles etc.Cell wall contains ergosterol .They are non motileFungi are chemoheterotrophs.(Require organic compounds for both carbon and energy sources.)They are osmiotrophic. They obtain their nutrition by absorption.Fungi may be unicellular or Multicellular.All fungi are Gram Positive.Most are aerobic and facultative anaerobic.Very few fungi are anaerobic.Reproduction may be sexual or asexual.

Many fungi reproduce both sexually and asexually.Food storage is generally in the form of lipids and glycogen.

Classification of fungi

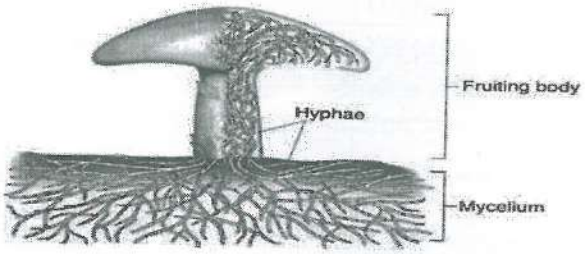
Fungi are classified based on hypha, spore, and reproduction.

There are four classes of fungi,

(a) Class Phycomycetes. (b) Class Ascomycetes.

(c) Class Basidiomycetes. (d) Class Deuteromyceters.

Fungus Structure



Fungi diseases

Mucomycosis

Candida auris

Valley fever (Coccidioldomycosis)

Ringworm

Candida albicans

Candida parapsilosis

Aspergillus Niger- Otitis disease

Fusariun

Exophiala

Dermatitis

Microsporiadia

7. Protozoa

They are acellular organism (consists of protoplasm).

Habitat: mostly aquatic, either free living or parasitic or commensal.

Body of protozoa is either naked or covered by a pellicle.

Locomotory organ are pseudopodia or cilia or absent.

Nutrition are holophytic or holozoic or saprophytic or parasitic.

Digestion is intracellular, occurs in food vacuoles.

Respiration: through the body surface.

Contractile vacuoles helps in osmoregulation.

Asexually reproduction is through binary fission or budding.

Sexual reproduction is by syngamy, conjugation.

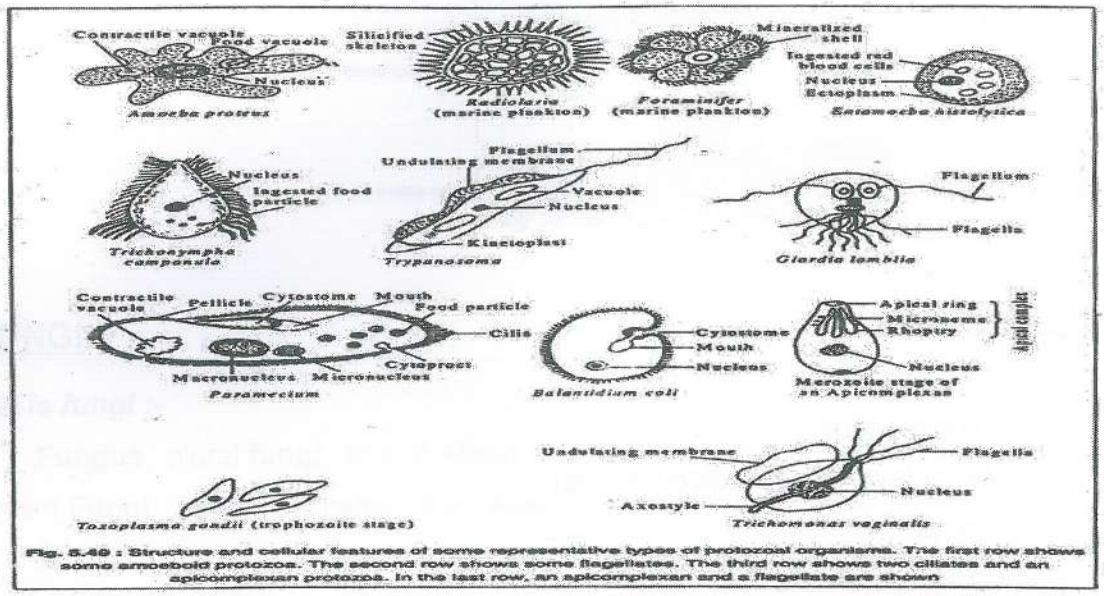


Fig. 5.49 : Structure and cellular features of some representative types of protozoal organisms. The first row shows some amoeboid protozoa. The second row shows some flagellates. The third row shows two ciliates and an apicomplexan protozoa. In the last row, an apicomplexan and a flagellate are shown

Some of the diseases caused by protozoa include: Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Amoebiasis	(*Entamoebahistolytica*)	Sewage, non-treated drinking water, flies in water supply, transfer from saliva.	Abdominal discomfort, fatigue, weight loss, diarrhea, bloating, fever
Cryptosporidiosis(oral)	Protozoan (*Cryptosporidium parvum*)	Collects on water filters and membranes that cannot be disinfected, animal manure, seasonal runoff of water.	Flu-like symptoms, watery diarrhea, loss of appetite, substantial loss of weight, bloating, increased gas, nausea
Cyclosporiasis	(*Cyclosporaayetanensis*)	Sewage, non-treated drinking water	cramps, nausea, vomiting, muscle aches, fever, and fatigue

Giardiasis (fecal -oral) (hand-to-mouth)	(*Giardia lamblia*) Most common intestinal parasite	Untreated water, poor disinfection, pipe breaks, leaks, groundwater contamination, campgrounds where humans and wildlife use same source of water. Beavers and muskrats create ponds that act as reservoirs for Giardia.	Diarrhea, abdominal discomfort, bloating, and flatulence
Microsporidiosis	Protozoan(*Microsporidia*),	*Encephalitozoonintestinalis* has been detected in groundwater, the origin of drinking water	Diarrhea and wasting in immunocompromised individuals.

What is sterilization :-

The Process of killing micro organisms including their spores called as sterilization. This is mainly two types

1) Physical Sterilization.
2) Chemical sterilization.

1)Physical Sterilization:-

In this sterilization by using sunrays & instruments like hot air oven, Autoclave, Pressure cooker..etc.

2) Chemical sterilization:-

It is the process of killing ,inhibition or removal of micro organisms by using of phenol , phenolic compounds, alcohols , halogens,aldehydes,hypochlorides etc.

8. Bacteriological analysis of drinking water by MPN method

What is mpn method :-

MPN Method means "**MOST PROBABLE NUMBERMETHOD**".

- MPN is most commonly applied for quality testing of water i.e *to ensure whether the water is safe or not in terms of bacteria present in it.*
- A group of bacteria commonly referred as fecal coliforms act as an indicator for fecal contamination of water.

Principle :

- Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas.
- The presence of bacteria is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube present in the **medium**.
- The number of total **coliformsis** determined by counting the number of tubes giving positive reaction *(i.e both color change and gas production).*
- comparing the pattern of positive results *(the number of tubes showing growth at each dilution)* with standard statistical tables.

In MPN method **MacConkey agarmedium** play very important role. Culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose).

At first by using of Hot air oven, sterilize all the glass ware tokillmicro organisms including their spores. Have on hand gloves & mouth mask to hygiene condition.

MPN test is performed in 3 steps :

In the multiple-tube method, a series of tubes containing a suitable selective broth culture medium (lactose-containing broth, such as MacConkey broth) is inoculated with test portions of a water sample. After a specified incubation time at a given temperature, each tube showing gas formation is regarded as "presumptive positive" since the gas indicates the possible presence of coliforms. However, gas may also be produced by other organisms, and so a subsequent confirmatory test is essential. The two tests are known respectively as the presumptive test and the confirmatory test.For the confirmatory test, a more selective culture medium (brilliant green bile broth) is inoculated with material taken from the positive tubes. After an appropriate incubation time, the tubes are examined for gasformation as before. The most probable number (MPN) of bacteria present can then be estimated from the number of tubes inoculated and the number of positive tubes obtained in the

confirmatory test, using specially devised statistical tables. This technique is known as the MPN method.

Equipment andCulture media / Reagent :

- Culture tubes containing inverted vials (Durham tubes): each tube should be large enough for a vial, completely filled with medium, to be submerged in it.
- Gas burner: a Bunsen or similar burner is adequate.
- Inoculation loop and holder: lengths of 24 or 26 gauge wire (7.5- 10 cm) should be used. Nichrome wire is acceptable, but platinumiridium is better. The wire is set in a handle made of metal or glass, of diameter similar to that of a pencil. To make the inoculation loop, the wire is bent to form a circle 3-4mm in diameter.
- Cleaning and maintenance equipment: items such as brushes for cleaning tubes, bottles, etc., a waste bin, and a tool kit are required.
- Safety equipment: there should be an adequate first-aid kit and a fire extinguisher or other means of fire control in every laboratory.

The following consumable items are required:

Culture media / Reagent : The following culture media and reagents are required

- MacConkey Broth with neutral red (double strength)
- MacConkey Broth with neutral red (single strength)
- Brilliant Green Bile broth (BGB)
- Tryptone water/Peptone water (for indole test)
- Kovac's
- Sample water to be tested.
- Hot air oven.
- Test tubes (3 sets of variouscapacitie and each set have 5 tubes).
- Test tube stands.
- Durham tubes.
- Auto clave.
- Hot plate.
- Water bath.
- Colony counter.
- Digital Balance.
- Laminar air flow

- Incubator
- Media (MacConkey Broth media- Double strength & Single Strength)
- EMB agar.
- Petri dishes
- Sterilized pipettes (10ml, 1 ml & 0.1 ml).
- Inoculation loop.
- Burner.
- New hand glows
- New mouth mask.
- Cotton (Absorbent & Non Absorbent).
- Laboratory disinfectant: for cleaning laboratory surfaces and the pipette discard bin.
- Detergent: for washing glassware, etc
- Sodium thiosulphate solution: required when chlorinated supplies are tested. Sodium thiosulphateneutralises any residual chlorine in samples at the time of collection, preventing it from acting on any micro-organisms present in water samples.
- Autoclave tape

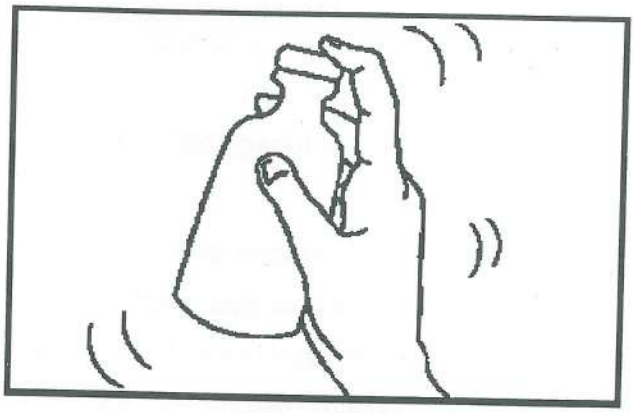
Culture media preparation

- Commercially available dehydrated media simplify the preparation of culture broths and are therefore recommended for laboratory work. Various manufacturers produce these media as powders, which can then be easily weighed, dissolved in distilled water, and dispensed into culture tubes before sterilisation.

- Preparation of media: Media should be prepared in accordance with the manufacturer's instructions, as follows: Dissolve the stated amount of the dehydrated medium in distilled water to obtain the double-strength or single-strength presumptive medium (MacConkey broth). The confirmatory medium (BGB) is required only in single-strength.
- Dispense the requisite volume into culture tubes containing an inverted Durham tube, and cap the culture tubes; simple cotton plugs may be used or more ideally metal slip-on caps may be used to cap the tubes.
- Sterilise in an autoclave at 115 °C for 10 minutes (or in accordance with the manufacturer's specifications). It is particularly important that media containing disaccharides, e.g. lactose, are not autoclaved at higher temperatures.
- The sterilised medium may be stored at room temperature (approximately 25°C) or, ideally, at 2-8°C. Media should in any case be warmed to room temperature

before use to ensure that all components have re-dissolved. In addition, since several dyes are light sensitive, the solution should be protected from exposure to light.

❖ **Procedure for inoculation of samples:** The procedure to be used for testing relatively unpolluted water, such as treated water from waterworks or distribution system or end user's tap, is described below (Figure 1.0.1).



1. **Presumptive test(stage – I):**

   - The presumptive test, is a screening test to sample water for the presence of coliform organisms.
   - If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction.
   - Take three sets of test tubes containing five tubes in each set
   - In three sets, take one set and transfer Double strength Media of 10 ml in each test tube of the set and drop the Durhum's in inverted position.
   - In the same way transfer of Single strength Mediaof 10ml to remaining two sets of each test tube with the inverted Durhum tube position.
   - Close the rim of three sets of test tubes with the non absorbent cotton tightly.
   - Arrange the three sets of test tubes in test-tube stand in proper manner and go for Autoclave.

Inoculation:

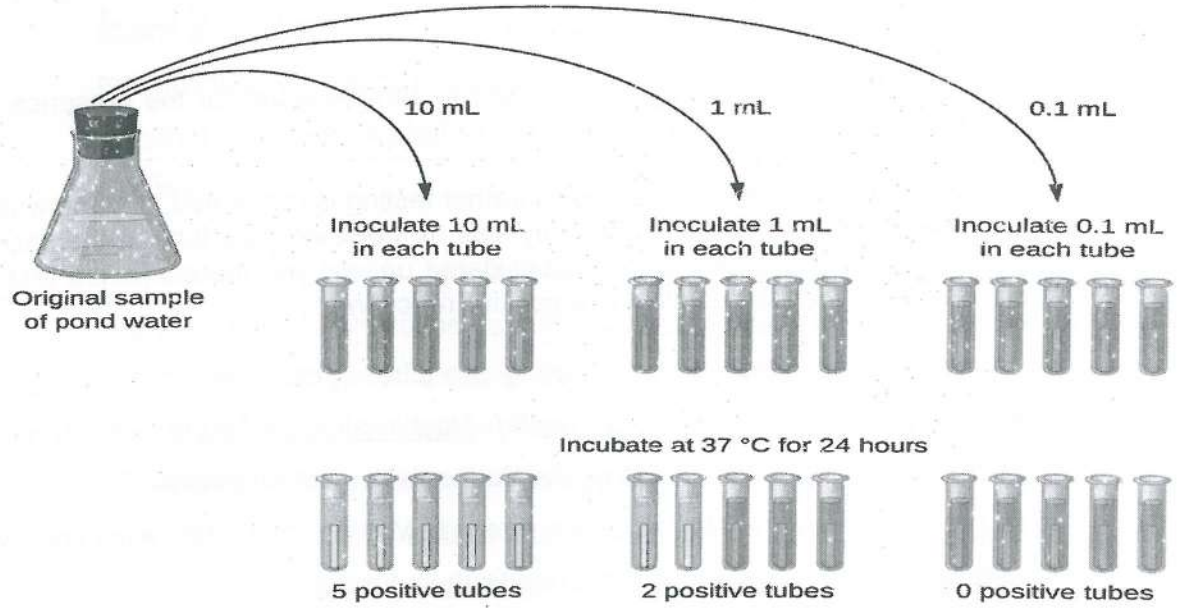
- Using 10 ml sterile pipette and transfer of 10 ml of sample to the each of double strength broth test tubes.
- In the same manner, using the 1 ml sterile pipette, transfer of 1 ml of sample to the one set of each of Single strength broth test tubes.
- In the same manner, using the 1 ml sterile pipette, transfer of 0.1 ml of sample to the one set of each of remaining Single strength broth test tubes
- Close the rim of three sets of test tubes with the non absorbent cotton tightly.

Incubation :

- Incubate the three sets of test tubes at 37^0C For 24 hours.

Observation :

- After incubation , observe the gas production in Durhum's tube and color change of the media
- Record the number of positive results from each set and compare with standard chart to give presumptive coliform count per 100 ml of water sample.



2. **Confirmatory test (stage -II) :**

The confirmatory test should be carried out at the end of both the 24-hour and the 48-hour incubation. Using a sterile loop, transfer one or two loops-full of sample, from each presumptive positive tube, into tubes containing Brilliant Green Lactose Broth (BGB). (Sterilize the inoculation loop before each transfer

by flaming and allow cooling.) The BGLB broth, called as confirmation broth, in addition to containing lactose also contains two components inhibitory to gram positive bacteria. Brilliant green is a dye related to crystal violet and belongs to thetriphenylmethane dye series. Oxgall is a surface active agent which also inhibits the growth of gram positive beacteria. Gas formation in 24 hours or 48 hours confirms the results of the presumptive step.

Procedure:

1. Add dehydrated ingredients to water ,mix thoroughly, and heat to dissolve.
2. Prepare fermentation tubes with 10 ml. BGLB medium along with Durham"s tubes. The numbers of tubes to be prepared are equal to all positive tests in the presumptive test.
3. Shake gently, the fermentative tubes with positive results and transfer one or two loops-full of medium in BGLB broth.Incubate the tubes at 35-37 °C for 48 hrs and record the tubes with gas formation as +ve number. This will be a better confirmation of Total Coliform bacteria.
4. The wrong concentration of BGLB or exposure of the media to excessive heat or light may give false positive resuts.
5. To confirm the presence of coliform bacteria, the completed test is carried out. For this test, inoculum form each positive tube of the confirmatory test is streaked on plate of EMB (EosinMethylen Blue) or Engo agar plate.
6. To confirm the presence of Thermotolerant coliforms, incubate the subculture tubes from each presumptive positive tube for 24 hours at 44.5±0.5°C (At the same manner, using the 1 ml sterile pipette, transfer of 1 ml of sample to the one set of each of Single strength broth test tubes.

Eosin MethylenBlue(EMB)agar plate counting):

- Measure the 3.6 grams of EMB media with the help of digital balance and dilute in 100 ml of distilled water and autoclave it .
- It is a selective stain for gram-negative bacteria.
- EMB contains dyes that are toxic to gram-positive bacteria.
- EMB is the selective and differential medium for coliforms. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1.
- Prepare petri dishes plate's with EMB agar media

Inoculation:-

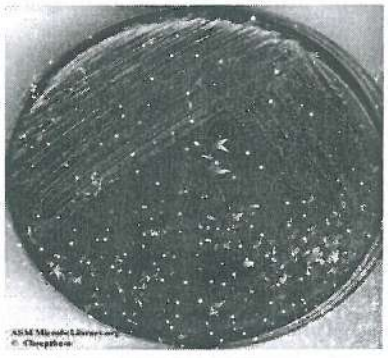
- Take the inoculum form each positive tube of the confirmatory test andthen inoculate with EMB agar petri dish plate with help of Inoculation loop.

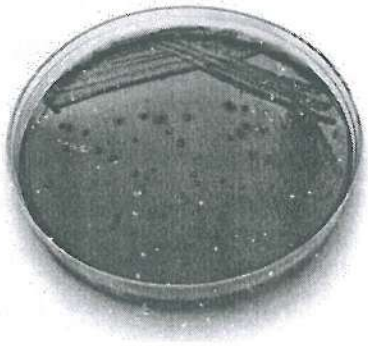
Incubation :-

- Incubate the EMB agar petri dish plate in inverted position in incubator at 37^0C for 24 hours.

Observation :-

- Look the typical colonies in the media ; blue black with **green metallic sheen** colonies are of **E.COLI** in EMB agar
- By using of Digital colony counter count the colony's on EMB agar plate.
- Then take average figure of colony count by standard formula.





3. Completed test/ Indole positive (Stage –III):

In completed test the characteristics of coliform colony and morphology of E.Coli is observed.

❖ Since some of the positive results from the confirmatory test may be false, it is desirable to do occasional completed test.

- Using a sterile loop, transfer one or two loops-full of sample, from each Brilliant Green Lactose Broth (BGB) positive tube, into tubes containing Peptone water or Tryptonewater.The numbers of Peptone water or Tryptone water tubes to be prepared are equal to all positive tests in the confirmative test.
- Incubate the broth cultures at 44.5^0C +/- 02^0C for 18 hrs to 24 hrs. .
- Examine the acid and Gas production in the tubes..

- To each tube of tryptone water, add approximately 0.2 mL of Kovacs reagent and mix gently.Formation of a brickredcoloured ring indicates positive indole test.
- The presence of indole is indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.
- The positive Indole test, gas production show the presence of E.Coli.
- Kovacs reagent is a biochemical reagent consisting of isoamyl alcohol, para-dimethylaminobenzaldehyde (DMAB), and concentrated hydrochloric acid

9. Faecal Coliforms (Thermotolerant Coliforms) MPN Test

The test is used to distinguish between total coliforms and faecal coliforms test using Escherichia coli medium (Table 6.7) is applicable for investigation of drinking water, stream pollution, raw water source, wastewater treatment systems, bathing water and general water quality monitoring

The term "faecal coliforms" has been used in water microbiology to denote coliform organisms that grow at 44 to 45.5°C and ferment lactose to produce acid and gas. So, thermotolerant coliform is more correct tem and is becoming more commonly used.

Before sterilisation, dispense fermentation tubes with sufficient medium to cover the inverted vials at least partially after sterilisation.

PROCEDURE OF FAECAL COLIFORMS TEST

1. Transfer all positive tubes from the total coliform MPN test (i.e. presumptive test) to Escherichia coli medium. This examination may be performed simultaneously with the confirmatory procedure using brilliant green lactose broth.

2. Use a sterile metal loop with minimum 30 – mm diameter to transfer the positive fermentation tube to Escherichia coli medium containing fermentation tube

3. Inoculated tubes are incubated at 44.5 ± 0.2°C for 24±2 h.

4. Place all Escherichia coli tubes in the water bath within 30 minutes after planting.

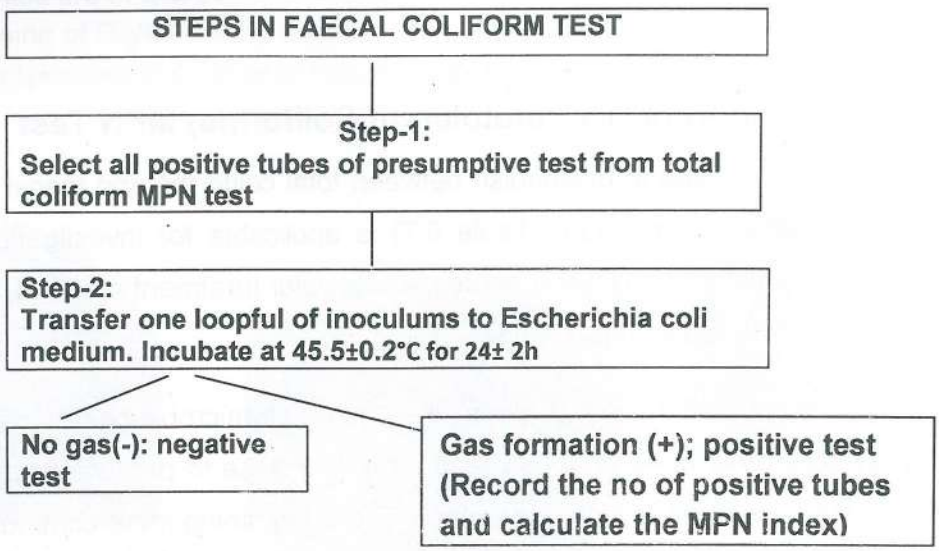
5. The water depth in the incubator should be sufficient to immerse tubes to the upper level of the medium.

Interpretation

Gas production within 24 h or less is considered a positive reaction indicating faecal origin. Failure to produce gas constitutes a negative reaction indicating a source other than the intestinal tract of warm – blooded animals.

RESULTS

Record the number of positive tube and refer Tables 6.10 or 6.11 for drinking water and Table 6.12 for effluent



Flow-diagram for conducting faecal coliform test.

10. Faecal Streptococci (FS) Test by MPN Method

The test is used to identify whether faecal contamination was from human origin or from warm – blooded animals origin. The presence of faecal streptococci is evidence of faecal contamination.

1. The normal habitat for faecal streptococci is the intestine of man and animals; thus, these organisms are indicator of faecal pollution.

2. FS tend to persist longer in the environment than thermotolerant or total coliforms and are highly resistant to drying.

3. The faecal streptococcus group consists of a number of species of the genus streptococcus, such as S. faecalis, S. faceium, S.avium,S. bovis, S.equinus, and S. gallinarum and all have been isolated from the faeces of warm-blooded animals.

4. The normal habitat of faecal streptococci is the gastrointestinal tract of warm-blooded animals.

5. Faecal Streptococci – two stains of faecal streptococci, namely –S. faecalis and S.faceium are the most human specific members of the FS group. The faecal streptococci are frequently used identification of faecal pollution in water. They are unable to multiply significantly in open water and do not survive long. Thus their presence in high numbers indicates recent pollution.

MPN Table:

MPNValues per 100ml. of sample and 95% confidence limits for various combinations of positive and negative results (when five 10ml,, five 1.0ml and five 0.1ml test portions are used).					
Combination of positives				**95% Confidence limits**	
10ml	**1.0ml**	**0.1ml**	**MPN Index/100ml**	**Lower**	**Upper**
0	0	0	<2	*	*
0	0	1	2	1.0	10
0	1	0	2	1.0	10
0	2	0	4	1.0	13
1	0	0	2	1.0	11
1	0	1	4	1.0	15
1	1	0	4	1.0	15
1	1	1	6	2.0	18
1	2	0	6	2.0	18
2	0	0	4	1.0	17
2	0	1	7	2.0	20
2	1	0	7	2.0	21
2	1	1	9	3.0	24
2	2	0	9	3.0	25
2	3	0	12	5.0	29
3	0	0	8	3.0	24
3	0	1	11	4.0	29
3	1	0	11	4.0	29
3	1	1	14	6.0	35
3	2	0	14	6.0	35
3	2	1	17	7.0	40

4	0	0	13	5.0	38
4	0	1	17	7.0	45
4	1	0	17	7.0	46
4	1	1	21	9.0	55
4	1	2	26	12	63
4	2	0	22	9.0	56
4	2	1	26	12	65
4	3	0	27	12	67
4	3	1	33	15	77
4	4	0	34	16	80
5	0	0	23	9.0	86
5	0	1	30	10	110
5	0	2	40	20	140
5	1	0	30	10	120
5	1	1	50	20	150
5	1	2	60	30	180
5	2	0	50	20	170
5	2	1	70	30	210
5	2	2	90	40	250
5	3	0	80	30	250
5	3	1	110	40	300
5	3	2	140	60	360
5	3	3	170	80	410
5	4	0	130	50	390
5	4	1	170	70	480
5	4	2	220	100	580
5	4	3	280	120	690
5	4	4	350	160	820
5	5	0	240	100	940
5	5	1	300	100	1300
5	5	2	500	200	2000
5	5	3	900	300	2900
5	5	4	1600	600	5300
5	5	5	>=1600	-	-

ANALYSIS OF WATER TREATMENT CHEMICALS

1. JAR TEST FOR DETERMINATION OF ALUM DOSAGE

Aim : To find the optimum coagulant dose in a water treatment system

Apparatus: Jar test apparatus, turbidity meter, pH meter, beaker, pipettes.

Reagent : 1% alum solution (dissolve 1.0 gram of alum in 100 ml distilled water)

Theory :Metallic salts hydrolyze in presence of the natural alkalinity to form metallic hydroxides. Metallic hydroxide are good adsorbents and hence can be removedassuspendedparticles by Alum, ferrous and ferric salts. The exact doses of these coagulants cannot be theoretically calculated and therefore, laboratory, tests have to be carried out using the Jar test. This enables the investigation of inter-related factors like pH, colour, turbidity, mineral, matter, temperature, time of flocculation and the degree of agitation, which control the coagulation and flocculation.

PROCEDURE:

1. Take 1 litre of sample into each of the beakers.
2. Add varying doses of alum solution i.e., 0.1, 1ml, 2ml, 3ml, 4ml or 6ml to different beakers simultaneously. One beaker is kept as blank in which no alum is added. These doses correspond to alum doses of 1mg/l, 10mg/l ,20mg/l, 30mg/l, 40mg/l and 60mg/l respectively. The dosage varies with the turbidity of water.
3. Switch on the motor and adjust the speed of paddles to about 100rpm so that rapid mixing of coagulant is done. Because of flash mixing, the positively charged aluminum ions will combine with the negatively charged turbidity impurities. Flash mixing (or rapid mixing) is done for 1 or 2 minutes.
4. Reduce the speed of paddles to about 30 to 40 RPM and continue mixing for 10 to 15 minutes. This corresponding to flocculating during which the particles already combined during coagulation combine to form much larger sized agglomerations of flock.
5. Switch off the motor and allow it to settle for 15 to 20 minutes. This corresponds to sedimentations or settling of impurities.

6. Collect the supernatant from each beaker with the help of a pipette without disturbing the sediment and find the turbidity of each.
7. Also record pH, colour, alkalinity and temperature of the supernatants as optimum coagulant dose depends on turbidity of raw water pH, alkalinity and temperature. Minimum alkalinity required for every 10mg/l dosage of alum is 4.5mg/l as $CaCO_3$. Deficiency must be supplemented with lime if the water has less alkalinity .Repeat the experiment at different pH values of the samples and at different doses of alum and find the optimum coagulant dose by measuring the turbidity in each case with the help of a nephelometer or any another turbidity meter. Note the ideal(or optimum)coagulant dose that correspondents to efficient and more economical removalof turbidity from water.

TABLE

Addition of 1ml of 1% alum solution to 1 litre of water corresponding to a dosage of 10mg/l.

Jar No. Sample	ml of 1 % alum Added	Dosage of alum mg/l	pH	Residual turbidity

Results and conclusion.

2. AVAILABLE CHLORINE IN SODIUMHYPO CHLORITE (NaOCl) SOLUTION

Principles:

The chlorine liberates iodine from potassium iodide (KI) solution at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulphate, using starch solution as indicator.

Apparatus:

1. Iodine flask 250ml -1no.
2. Burette -50ml
3. Pipette
4. Measuring cylinder 200ml – 1no.

Reagents:

a. Acetic acid(conc.)

b . Potassium Iodide Crystals

c . Standard Sodium Thiosulphateof 0.1N

d. Starch Indicator Solution : Boil 100ml distilled water take 5gms starch and make a paste with boiled distilled water gradually transfer all the paste to boiled distilled water.

Procedure:

Take 50ml.of the sample in Iodine flask , add 2to 3 grams of Potassium Iodide crystals, 10 ml. of Glacial Acetic acid. Titrate with 0.1 N standard sodium thiosulfate solution,until the Iodine colour is nearly gone, then add 1ml. of starch indicator solution and complete the titration to the disappearance of the Blue colour.

Available chlorine (as Cl^-) percent mass by volume:(AXNX0.3546)X100/V

Sodium Hypochlorite as (NaOCl), percent mass by volume :(AXNX0.3722)X100/V

3. AVAILABLE CHLORINE IN BLEACHING POWDER

Reagents:

1. Glacial acetic acid
2. Potassium iodide Crystal
3. Sodium thiosulphate 0.1N
4. Starch indicator.

Theory: Chlorine is a strong oxidizing agent and liberates iodine from iodide ion.

$Cl + 2KI \longrightarrow I2 + 2Cl$

Starch gives blue colour with iodine

The liberated iodine is treated with standard Sodium Thiosulphate – a reducing agent

$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$

The disappearance of blue colour indicates the completion of reaction with free iodine which is converted back to iodine by $Na_2S_2O_3$.

PROCEDURE

1. Take 2. 5gms (accurately weighed) of fresh bleaching powder adding small quantity of water to it and prepare fine paste. Add some more water, stir and allow to settle for a few minutes. Dilute it with distilled water to make up to 250ml and stopper the container.
2. Take 25ml of samplefrom above bleaching powder solution in a conical flask and 2 to 3 gms. of Potassium Iodide
3. Add 100ml of distilled water and 10ml of acetic acid and allow the reaction to complete in dark
4. Titrate the free-iodine liberated sampie with standard 0.1 N sodium thiosulphate solution until the yellow colour of the liberated iodine is almost faded out.
5. Add 1ml of starch solution and continue the titration until the blue colour disappears.
6. Note down the quantity of sodium thiosulphateadded(V).

Calculation:

Available Chlorine percentage by mass:VXN X 35.46/M

V: Volume of the standard Sodium thiosulfate Solution

N: Normality of the standard Sodium thiosulfate Solution

M: Mass cf the Bleaching powder taken for the test.

DRINKING WATER SPECIFICATION IS 10500:2012

Table 1 Organoleptic and physical parameters

Sl. No	Characteristic	Requirement (Acceptable Limit)	Permissible limit in the absence of alternative source	Method of test,Ref to part of IS 3025	Remark
1	2	3	4	5	6
1	**Colour Hazen units, Max**	5	15	Part 4	Extended to 15 only, if toxic substances are not suspected in absence of alternate sources
2	**Odour**	Agreeable	Agreeable	Part 5	a) Test cold and when heated, b) Test at several dilutions
3	**pH**	6.5 to 8.5	No relaxation	Part 11	-----
4	**Taste**	Agreeable	Agreeable	Parts 7 and 8	Test to be conducted only after safety has been established
5	**Turbidity, NTU, Max**	1	5	Part 10	----
6	**Total dissolved solids, mg/l,Max**	500	2000	Part 16	----

NOTE — It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 2 General Parameters Concerning Substances Undesirable in Excessive Amounts

1	Aluminium (as Al), mg/l, Max	0.03	0.2	IS 3025 (Part 55)	----
2	Ammonia (as total ammonia-N),mg/l,Max	0.5	No relaxation	IS 3025 (Part 34)	----
3	Anionic detergents (as MBAS),mg/l,Max	0.2	1.0	Annex K of IS 13428	----
4	Barium (as Ba), mg/l, Max	0.7	No relaxation	Annex F of IS 13428*or IS 15302	----
5	Boron (as B), mg/l, Max	0.5	1.0	IS 3025 (Part 57)	----

6	Calcium (as Ca), mg/l, Max	75	200	IS 3025 (Part 40)	----
7	Chloramines (as Cl2), mg/l, Max	4.0	No relaxation	IS 3025 (Part 26)*or APHA 4500-Cl G	----
8	Chloride (as Cl), mg/l, Max	250	1000	IS 3025 (Part 32)	----
9	Copper (as Cu), mg/l, Max	0.05	1.5	IS 3025 (Part 42)	----
10	Fluoride (as F) mg/l, Max	1.0	1.5	IS 3025 (Part 60)	----
11	Free residual chlorine, mg/l, Min	0.2	1.0	IS 3025 (Part 26)	To be applicable only when water is chlorinated. Tested at consumer end. When protection against viral infection is required, it should be minimum 0.5 mg/l
12	Iron (as Fe), mg/l, Max	0.3	1.0	IS 3025 (Part 53)	Total concentration of manganese (as Mn) and iron (as Fe) shall not exceed 0.3 mg/l
13	Magnesium (as Mg), mg/l, Max	30	100	IS 3025 (Part 46)	
14	Manganese (as Mn), mg/l, Max	0.1	0.3	IS 3025 (Part 59	Total concentration of manganese(as Mn) and iron (as Fe) shall not exceed 0.3 mg/l
15	Mineral oil, mg/l, Max	0.5	No relaxation	Clause 6 of IS 3025 (Part 39) Infrared partition method	----
16	Nitrate (as NO3), mg/l, Max	45	No relaxation	IS 3025 (Part 34)	----
17	Phenolic compounds (as C6H5OH),mg/l ,Max	0.001	0.002	IS 3025 (Part 43)	----
18	Selenium (as Se), mg/l, Max	0.01	No relaxation	IS 3025 (Part 56) or IS 15303*	----
19	Silver (as Ag), mg/l, Max	0.1	No relaxation	Annex J of IS 13428	----
20	Sulphate (as SO4) mg/l, Max	200	400	IS 3025 (Part 24)	May be extended to 400 provided that Magnesium does not exceed 30
21	Sulphide (as H2S), mg/l, Max	0.05	No relaxation	IS 3025 (Part 29)	----

22	Total alkalinity as calcium carbonate, mg/l, Max	200	600	IS 3025 (Part 23)	----
23	Total hardness (as CaCO3),mg/l, Max	200	600	IS 3025 (Part 21)	----
24	Zinc (as Zn), mg/l, Max	5	15	IS 3025 (Part 49)	----

Note :

1 In case of dispute, the method indicated by '*' shall be the referee method.

2 It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 3 Parameters Concerning Toxic Substances

1	Cadmium (as Cd), mg/l, Max	0.003	No relaxation	IS 3025 (Part 41)	----
2	Cyanide (as CN), mg/l, Max	0.05	No relaxation	IS 3025 (Part 27)	----
3	Lead (as Pb), mg/l, Max	0.01	No relaxation	IS 3025 (Part 47)	----
4	Mercury (as Hg), mg/l, Max	0.001	No relaxation	IS 3025 (Part 48)/Mercury analyzer	----
5	Molybdenum (as Mo), mg/l, Max	0.07	No relaxation	IS 3025 (Part 2)	----
6	Nickel (as Ni), mg/l, Max	0.02	No relaxation	IS 3025 (Part 54)	----
7	Pesticides, µg/l, Max	See Table 5	No relaxation	see Table 5	----
8	Polychlorinated biphenyls, mg/l,Max	0.0005	No relaxation	ASTM 5175*or APHA 6630	----
9	Polynuclear aromatic hydro-carbons (as PAH), mg/l, Max	0.0001	No relaxation	APHA 6440	----
10	Total arsenic (as As), mg/l, Max	0.01	0.05	IS 3025 (Part 37)	----
11	Total chromium (as Cr), mg/l, Max	0.05	No relaxation	IS 3025 (Part 52)	----
12	**Trihalomethanes:**				----
	a) Bromoform, mg/l, Max	0.1	No relaxation	ASTM D 3973-85* or APHA 6232	----
	b) Dibromochlorometh ane, mg/l, Max	0.1	No relaxation	ASTM D 3973-85* or APHA 6233	----

	c) Bromo dichloromethane, mg/l,Max	0.06	No relaxation	ASTM D 3973-85* or APHA 6234	----
	d) Chloroform, mg/l, Max	0.2	No relaxation	ASTM D 3973-85* or APHA 6235	----
Note:					
1 In case of dispute, the method indicated by '*' shall be the referee method.					
2 It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.					
Table 4 Parameters Concerning Radioactive Substances					
	Radioactive materials:				
1	a) Alpha emitters Bq/l, Max	0.1	No relaxation	Part 2	----
	b) Beta emitters Bq/l, Max	1	No relaxation	Part 1	----
Note : It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.					

Online water quality monitoring with real time data transfer by using Internet of Things(IOTS):

In some difficult site conditions in rural habitations, manual disinfection operations in water supply systems (viz., mixing of conventional liquid Chlorine or Bleaching powder in reservoirs and in distribution line),at regular time intervals, may not be practically possible.

Keeping in view of the difficulties in manual mixing of the disinfectants and monitoring of disinfection in the water supply system, the Govt. of A.P. is making efforts to explore the new technologies of end to end Water Disinfection Systems for community water distribution systems in rural habitations.

1. OZONE based online Disinfection System :

The Eco Smart Systems, Baroda, has developed OZONE based online Disinfection System (which uses Ozone gas as disinfectant) for water supply systems in Multi Village Schemes (MVS) and single village Schemes in rural habitations.
The entire system consists of three units Viz.,

A. Ozone Generator: It consists of the following subunits.

i. Ozonator .
ii. Oxygen concentrator .
iii. Water chiller
iv. Venturie system
v. Ozone injection skid

B. Online Ozone Residual Monitoring Unit:

This online residual ozone monitoring unit is based on "Indigo Tri-sulfonate" Method. The features of the unit are,
Display Range: 0-200.0 PPB, 0-2.000 PPM
Accuracy: ± 0.02 PPM

C. Web/Mobile application based online monitoring system which gives the dosage of ozone at the source(i.e, at Head works), and at different points in the distribution system through mobile app.

2. Online Liquid Chlorine dosage and Web monitoring System

M/s PranayEnterprises , Hyderabad, has developed Online Chlorine Dosing with Web monitoring System

A. Online Chlorine Dosing System:

The Chlorine is added in the form of Liquid Chlorine (Sodium Hypo chlorite) with 12.50 – 15.00 % weight / volume of available Chlorine), which is stored in the air tight plastic drum, with protection from sun light. By adjusting the dosage(1% to 100%), the chlorine level,in OHSR water, is maintained at 0.5 – 0.7 ppm. The dosing system works with or without electric power. The Liquid chlorine, is Grade –II of IS 11673 : 1992, with Drug Licence.

B. Online chlorine monitoring system with Web based Internet application.

The residual Free Chlorine, in the supplied water, in one of the distribution line, is being measured continuously, through Chlorine sensing electrode, and the Chlorine levels are being monitored through Web based internet application.

3. Expert 365:Disinfection through Ionization& UV with online monitoring:

Expert 365 provides an end to end solution for disinfection of the water supply systems, with online monitoring of 40 drinking water parameters and Mobile Application based information system.

The system consists of four units, viz.,

a. ION 365 (Disinfection unit):

This disinfection unit consists of One UV lamp and an ionization device. The Ionization device consists of two electrodes (viz., Copper and Silver), which continuously releases copper and silver ions in the water. The nascent copper and silver ions are known to have a property to disinfect the water. Both UV lamp and Ionization device are dipped in the water.

b. WATER 365 (Water quality monitoring):

It is Water Quality Monitoring Unit. It monitors the 6 Physico- chemical parameters, 12 Health & Aesthetic parameters , 17 metal and 7 Ionic species in the drinking water at pre- defined regular intervals and send the test results to concerned officers through Mobile Application.

Amount of disinfectants, to be added in drinking water to get 0.75 ppm of available chlorine at Head Works (as per WHO guidelines)		
Water in M3 (1 M^3=1000 lits.)	Calcium Hypo Chrlorite (Bleaching powder :) 30.00% available Chlorine. (in Gms,)	Sodium Hypochlorite Solution (Grade - I) Available Chlorine (as Cl^-) percent mass by volume. (in ml.)
1	2.5	15
1.2	3	18
1.5	3.75	22.5
2	5	30
2.5	6.25	37.5
3	7.5	45
4	10	60
5	12.5	75
6	15	90
7	17.5	105
8	20	120
10	25	150
12	30	180
15	37.5	225
20	50	300
30	75	450
40	100	600
50	125	750
60	150	900
70	175	1050
100	250	1500
120	300	1800
150	375	2250
200	500	3000
250	625	3750
300	750	4500
400	1000	6000
500	1250	7500

Dosage Calculations:

Bleaching Powder (Grade I: 34% and Grade II : 32% of available Chlorine)

Let us assume that the average available chlorine is 30% (loss of 2 to 4% during the storage conditions)

i.e. 100 grams of Bleacing powder contains 30 gms of available chlorine.

i.e, 2.5 gms of Bleacing powder contains 0.75 gms of available Chlorine.
As per WHO guidelines, the available chlorine shall be 0.75 ppm (i.e., 0.75 mg./lit.) at head works.

Hence, in 1 lit. waterwater shall contain 0.75 ppm (0.75 mg.) of available Chlorine.

1000 lits.(1M3) of water shall contain : 0.75 mg X1000 = 7.5 gms. of available chlorine.

Hence to get 0.75 ppm of available chlorine in 1000 lits, we have to add 2.5 gms of bleaching powder.

2. Sodium Hopy Chlorite Solution (Grade -I : 4-6%mass by volume):

Let us assume that the average available chlorine in SodiumHypochlorite, is 5% (loss of 1 during the storage conditions)

i.e. 100 lits. ofSodiumHypo Chlorite solution contains 5 gms of available chlorine.

i.e, 15 ml of Sodium Hypo Chlorite contains 0.75 gms of available Chlorine.
As per WHO guidelines, the available chlorine shall be 0.75 ppm (i.e., 0.75 mg./lit.) at head works.

Hence, in 1 lit. waterwater shall contain 0.75 ppm (0.75 mg.) of available Chlorine.

1000 lits.(1M3) of water shall contain : 0.75 mg X1000 = 7.5 gms. of available chlorine.

Hence to get 0.75 ppm of available chlorine in 1000 lits , we have to add 15 ml of Sodium Hypo Chlorite solution.

In- Plant water testing Laboratories at Head works of Water Treatment Plants.

I. Tests to be conducted at the In- plant Laboratories:

1. **pH:** pH is an important parameter to be measured in the raw water and during the flocculation and coagulation of the Raw water. pH should be 6.5-7.5 to achieve maximum coagulation and flocculation of Raw water. The dosage of Alum also depends on the pH of the raw water to some extent.

2.**Turbidity** : Turbidity of treated water should be <5 NTU (Nephlometric Turbidity units.) During the coagulation, flocculation and sedimentation, turbidity of the raw water will be removed substantially.

3. Suspended Solids: All the Suspended solids(measured in mg./lit.) will be settled down during settlement.

4. **Total Dissolved Solids (TDS)**: Total Dissolved Solids (measured in mg./lit.) indicates the load of salts dissolved in the water.

5. **Jar Test for Alum Dosage.:** Through the JAR Test , the alum dosage(mg./ lit.) can be fixed for coagulation and Flocculation process.

6. **Chlorine Demand.** Chlorine demand (in mg./lit) in the raw water after filtration, will be useful to fix the dosage of disinfectant chemicals (viz., Bleaching powder/ liquid chlorine etc.,)

7. **Residual Free Chlorine:** The residual free chlorine (measured in mg./lit.) in the treated water should be 0. 2 mg./lit (min.) at tail end point.

II. Instruments Required:

1. pH meter.
2. Conductivity meter.
3. Turbidity meter.
4. Chloroscope.
5. Flocculator (for JAR test)

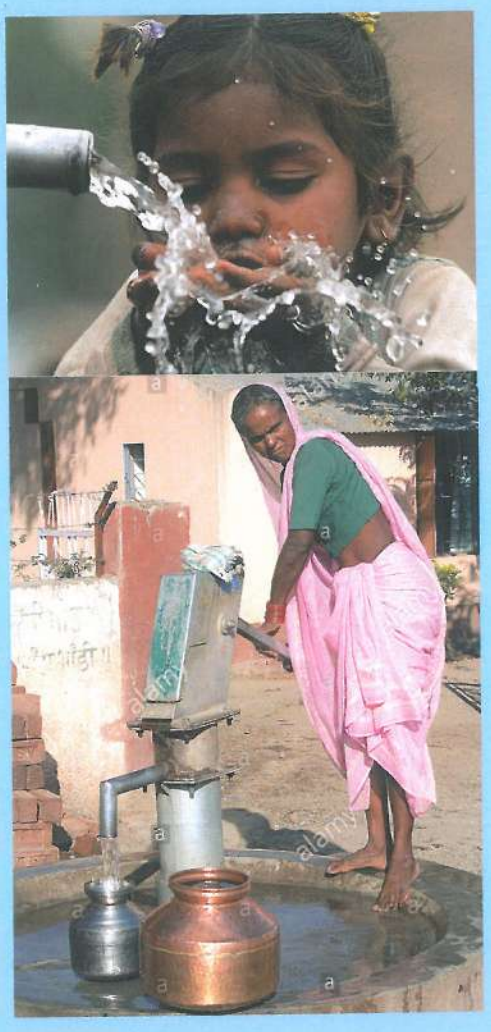
III. Frequency of Testing:

1. Raw Water: Two times for ph, Jar Test and Turbidity.
2. At Coagulation unit: Four times for pH and Turbidity.
3. At Flocculation unit: Two times for pH and Turbidity.
4. At Sedimentation unit: Two times for pH and Turbidity.

5. At Filtration unit: Two times for pH and Turbidity.

6. Treated Water : Two times for Turbidity, and Residual free chlorine.

Note: Samples should be taken at the exit points of every unit.



ISSUED BY

THE PROJECT DIRECTOR,

STATE WATER & SANITATION MISSION

RURAL WATER SUPPLY & SANITATION DEPARTMENT

VIJAYAWADA