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LABORATORY MANUAL ON WATER ANALYSIS

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TECHNOLOGY MISSION ON
DRINKING WATER IN VILLAGES
AND RELATED WATER MANAGEMENT

LABORATORY MANUAL ON WATER ANALYSIS

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INTRODUCTION

An initial approach to define a problem in environmental sciences, necessitates the use of analytical methods & procedures in the field and laboratory that have been proved to yield reliable results. Once the problem is defined, then only satisfactory solution can be sought out. Numerous analytical methods have been developed to obtain the factual information required to find out a solution of problems as well as to have constant supervision to maintain economical and satisfactory performance of the treatment facilities constructed.

Analytical procedures, a need to obtain quantitative information, are often a mixture of chemical, Biochemical Biological, Bacteriological, Bioassay and instrumental methods and interpretation of the data is usually related to the effect on microorganisms or human beings. Also many of the determinations fall under the micro-analysis because of the small amounts of contaminants present in the sample.

More emphasis has been given to cover all parameters under different analytical procedures which are prerequisite for assessment and control of water quality. In many cases different methods were proposed for the same determinant, only those which can be employed for routine analysis of water samples are discussed in detail.

To enable comparison of results by different analysts it is important to carry out determinations by standard methods.

General information

Collect the sample along with the suspended matter. Shaking may be required before taking an aliquot for analysis. Use a cylinder (100 ml) or a sawed off pipette when necessary.

Calibrate all the glass-wares.
Use A.R. grade reagent.

Isolate poisonous substances and mark them as poison.

Express the results as milligrams of parameter per litre of sample. Round off the digits which are not significant.

Statistical analysis of the data should be done before any conclusion is drawn.

In water samples the sum of anions (me/l) is equal to sum of cations (me/l) within reasonable limits if the ionic concentration is not excessive. This balance is not possible for polluted samples.

SAMPLING

INTRODUCTION

The collection of water samples may seem a relatively simple task. However to obtain representative water samples and to preserve their integrity until they are analysed in the laboratory requires a series of steps, procedures and practices. A representative sample can easily be obtained from rivers and lakes which are relatively homogeneous, whereas many water-bodies have significant spatial and temporal variations and the collection of a representative sample becomes much more complex.

The objective of the sampling is to collect a portion of material small enough in volume to be conveniently transported to and handled in the laboratory while still accurately representing the material being sampled. This implies, first, that the relative portions of the concentrations of all pertinent components must be the same in the samples as in the material being sampled and second, that the sample must be handled in such a way that no significant changes in composition occurs before the tests are performed. The analysis is generally intended to reveal the composition of the waters at the time or over the period of sampling. Consequently errors are introduced if changes take place between taking of the sample and analysis being carried out. There is infact, a strong likelyhood that such changes will occur in most of the waters. The arrangement should be such that these are prevented or at least minimised.

PLANNING OF SAMPLING

The monitoring of water quality to give reliable and usable data requires that analytical and other resources are employed to the best advantage. The first step in the planning of water monitoring is to decide what data is needed and how it is useful. The type of investigation, purpose of study and anticipated variations are other points to be considered. The first stage of planning of the sampling programme is the selection of the most suitable site to provide the required data.

SITE SELECTION :

The objectives of water quality monitoring system are

1) To assess the impact of activities by man upon the quality of water and its suitability for required uses
2) To determine the quality of water in it's natural state which might be available to meet the future needs and
3) To keep under observation the sources and path way of specified hazardous substances

The selection of sampling site is decided by the various uses of the water and by their location, relative magnitude and importance. The chances of accidental pollution is also an important factor and should be considered. The location of a river used down stream of large urban or industrial area, imposes greater risk and requires more supervision than similar uses located upstreams.

TYPE OF SAMPLES :

1. Grab or catch samples :

A sample collected at a particular time and place can represent only the composition of the source at that times and place. However, when a source is known to be fairly constant in composition over a considerable period of time or over substantial distances in all direction, then the sample may be said to represent a larger time period or larger volume or both, than the specific point at which it was collected. In such circumstances same source may be quite well represented by single grab samples. When a source is known to vary with time, grab samples collected at suitable intervals can be of great value in documenting the extent, frequency, and duration of these variations. In case the composition of a source varies in space rather than in time, a set of samples collected from appropriate locations with less emphasis on timing may provide the most useful information.

2. Composite samples :

The term composite refers to a mixture of grab samples collected at the same sampling point at different time. Sometimes the term time composite is used when it is necessary to distinguish this type of sample from other. Time composite samples are most useful for observing average concentrations, as an alternative to the separate analysis of a large number of samples, followed by computation of average and total results. A composite sample of 24 hr. period is considered standard for most determinations. Composite samples cannot be used for determinations, of components or characteristics subject to significant and unavoidable changes on storage.

3. Integrated samples :

Mixture of grab samples collected from different points simultaneously or as nearly as possible is called integrated sample. Such samples are useful for river or stream that varies in composition across its width and depth. The need for integrated samples also may exist if combined treatment is proposed for several separate waste water stream. The preparation of integrated samples requires special equipment to collect samples from a known depth, without contamination by over lying water. Prior knowledge about volume, movement and composition of the various parameters of the water being sampled is also required.

SAMPLING FREQUENCY :

Water samples should be collected at intervals so that no change in quality could pass unnoticed. The quality of water in various water bodies is rarely if ever constant in time but is subjected to change.

The larger the number of samples from which the mean is derived, the narrower will be the limits of the probable difference between observed and true values. However, the sampling schedule is a compromise between accuracy and the funds, personnel for the work.

NUMBER OF SAMPLES :

Number of samples and how often should samples be collected, are calculated by statistical considerations. The following frequencies of sampling may be adopted provisionally.

a) Weekly samples for one year
b) Daily samples for 7 days consecutively (4 times/year)
c) Round the hour sampling for 24 hours
d) 4-hourly samples for 7 days - and 4 times/year

The parameters may be limited during these samplings but should be pertinant to the source/sampling station.

The analytical data collected as per the above procedure will help to lay proper emphasis on parameters of relative importance; their ranges, interferences, and frequencies of their occurrence. This is applicable to rivers, lakes, industrial effluent outfall.

SAMPLE CONTAINERS :

It is advantageous to measure the quantity of water in situ by means of sensors which are lowered into position rather than by withdrawing samples. However, it is not always possible. Water samples are, therefore, collected in suitable containers. A sample container must satisfy the following requirements.

1. It could easily be freed from contamination.
2. It should not change the relevant water characteristics.
3. It should have adequate capacity for storing the samples.
4. It should be resistant to impact and to internal pressure which is increased by expansion of water or by release of dissolved gases at elevated temperature on storage.

The sample bottle may be made of either glass or plastic usually polyethylene. It must be capable of being tightly sealed either by stopper

or cap. The bottles should be soaked with 10% HCl for 24 hours and then thoroughly cleaned and rinsed with distilled water.

The specific situation will determine the use of the bsgh or pec (bsgh-Borosilicate glass bottle, pec-polyethylene container). Rinse with chromic acid solution (35 ml saturated $Na_2Cr_2O_7$ in 1 l conc H_2SO_4) followed by tap and distilled water and then invert them to dry.

SAMPLING EQUIPMENT :

Numerous types of sampling equipments have been devised. However, the sampler design is generally immaterial except for dissolved gases or constituents particularly affected by atmospheric gases. Sampling equipments are briefly described below:

Grab Samplers :

Grab samplers can be divided into discrete (surface or sp. depth) and depth integrating samplers. A grab sample may be taken using a "Sampling iron" with a appropriate bottle or a pump type sampler. Composite samples can be made from several grab samples mixed in equal proportions or in proportion according to the flow at the time of sampling.

Depth integrating samplers :

This apparatus is a device which is made of iron and painted with a rust inhibitor. The weight of the sampler is approximately 2.7 kg (Fig. 1). Typically, design permits the use of 2-L sample bottle when the bottle neck holder is in the upper position, smaller bottles may be used when the holder is located in lower position.

The sample bottles are placed in the sampler and secured by the neck holder. In some cases, sampling irons may have provision of additional weights to ensure a vertical drop in the strong currents. A depth integrated sample is taken by permitting the sampler to sink to the desired depth at a constant rate and then retrieving it at approximately

the same rate. The rate should be such that the bottle has just been filled when reaching the surface.

Discrete samplers :

Discrete samplers are used to collect water at specific depth. An appropriate sampler is lowered to the desired depth, activated and then retrieved (Fig.2). The Kemmerer style sampler is one of the oldest types of messanger operated vertical samplers. The samples should be taken at a known depth and without aeration. Depth samples can be collected with a bottle which can be closed by stopper, controlled by a cord permitting samples to be taken at any given depth. The bottle is required to be clamped into a weighted container. The total weight to be lifted out of the water will be atleast twice that of the sample.

Special samplers for Dissolved oxygen (D.O.) sampling are available. However, the discrete sampler is adequate for D.O. Samples. Sampling of shallow streams is not advisable with this sampler; in such case, gently tilt the bottle downstream, minimizing sample agitation (bubbling).

OTHER POINTS REQUIRING ATTENTION ARE :

Sample where water is well mixed. Weirs enhance the settling of solids upstream and accumulate floating solids and oil downstream, therefore, such location should be avoided as a sample source.

Avoid large non-homogenous matter such as leaves, rags, twigs and other floating material in the sample.

Sample facing upstream to avoid contamination by slowly drawing water from the source into the container.

Force sampling container through the entire cross saction of the stream, whenever possible.

Ascertain that the sampler operates at the proper time before the sampling with a depth sampler. If doubt exists, discard & resample.

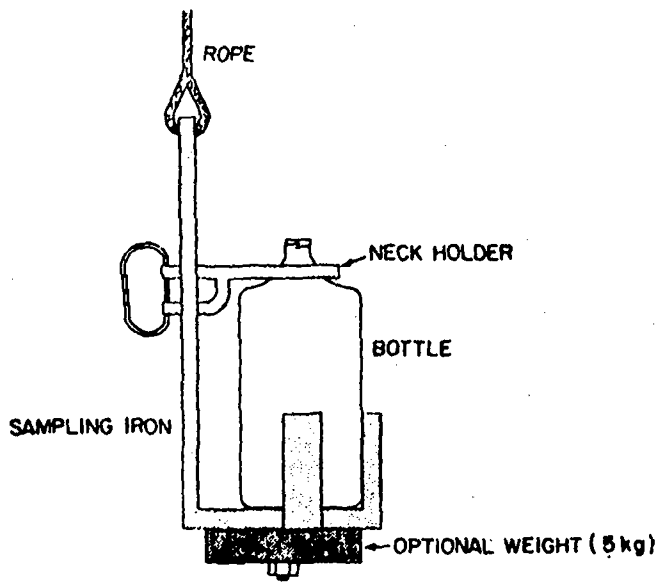


Fig.1: Depth Integrating Sampler

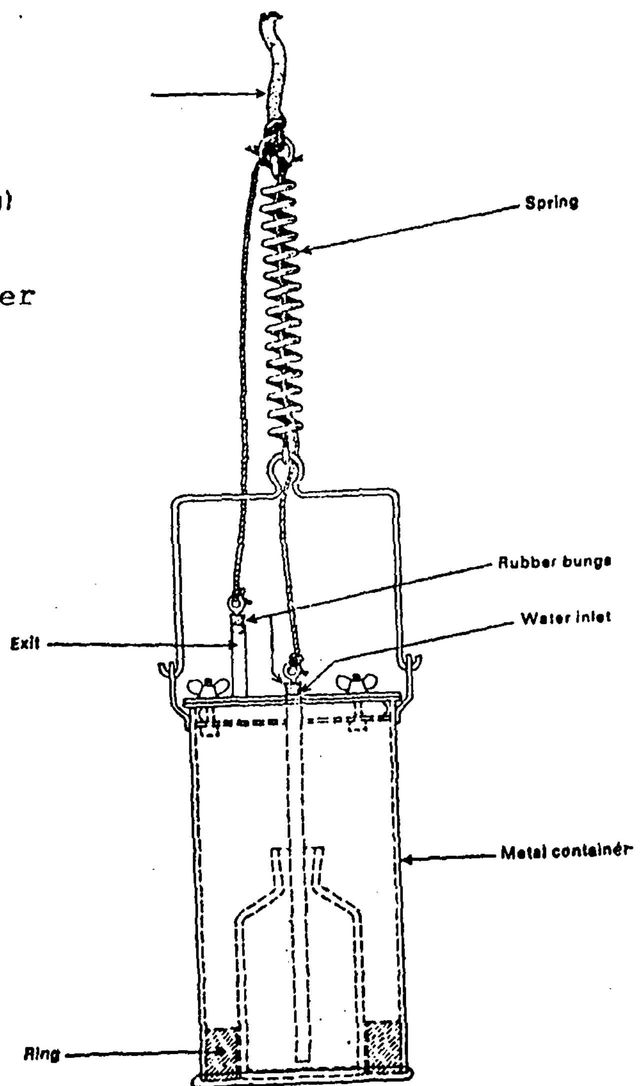


Fig.2: Discrete Sampler

The schedules of the factory for waste discharges must be known in order to avoid the sample from a batch dumping.

Provide complete information on the source & conditions under which the sample was collected.

Attach a record tag on the sample container. The tag should contain information on sample number source of sample, analysis required, temperature and name of person taking the sample. The tag should be signed, time and date should be recorded by the person taking the sample.

FIELD MEASURED PARAMETERS

A number of parameters including pH, conductivity, dissolved oxygen, ammonia, CO_2, temperature, turbidity and residual chlorine should be measured at the sampling site immediately after collection of sample. However in situ measurements of these parameters are recommended.

PHYSICO-CHEMICAL ANALYSIS

INTRODUCTION

Physico-chemical analysis is the prime consideration to assess the quality of water for its best usage say for drinking, bathing, industrial processing and so on, while for waste water either domestic or industiral to know the pollutional strength and its effect on the ecology.

River water often necessitates examination of water samples from different points and under varying conditions to find out the extent of pollution and purification that takes place in the water.

Well waters are examined to locate the potable source of water as well as to study the effect of pumping in coastal areas, or in saline water.

Waters are also examined to test the samples to ascertain their suitability for potability or a particular trade, e.g. paper making, tanning, steam raising, dying, dairying etc. In such case, a particular parameter assumes importance e.g. for steam raising water should be checked for hardness and dissolved oxygen, water used in textiles should be checked for iron and hardness.

Similarly domestic and industrial waste waters are also analysed for various parameters to decide upon what physical, chemical or biological treatment should be given to make them suitable for discharge either on land for irrigation or in other water bodies.

Comparatively this analysis for example, determination of pH, temperature, DO, can be done quick enough to be adopted by regulatory agencies to monitor and control the ecological balance of nature.

TEMPERATURE

Measurement of temperature is an important parameter required to get an idea of self purification of rivers, reservoirs and control of treatment plant. Water temperature is also important parameter for fish life. It is the important factor for calculating the solubility of oxygen and the carbon dioxide, bicarbonate and carbonate equilibrium. The temperature of drinking water has an influence on its taste.

APPARATUS

A thermometer having a quick response, with 0.1°C division, checked against a precision thermometer.

PROCEDURE

Immerse the thermometer directly in the water body for a period of time sufficient to permit constant reading. If it is not possible to take reading directly,then collect water in a sampling bottle, nearly one litre, and measure the temperature by dipping the thermometer in the sample. But while collecting the sample, it must not be exposed to heat or direct solar radiation. Tap water temperature should be measured in a bottle through which the water flows until a constant reading is reached.

Record temperature in celsius scale to the nearest 0.1°C.

COLOUR

When viewed by transmitted light through a depth of several feet, pure water exhibits a light blue colour which may be altered by the presence of organic matter to greenish blue, green, greenish yellow, yellow or brown. Colour is removed to make a water suitable for general and industrial applications. Coloured industrial waste water may require colour removal before discharge into water courses.

The term 'Colour' used here is true colour, that is the colour of 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. In some highly coloured industrial waste waters, colour is contributed principally by colloids or suspended materials. In such cases both true colour and apparent colour should be determined.

The optimal method for removing turbidity without removing colour has not been found yet. Filtration yields results that are reproducible from day to day and among laboratories. However, some filtration methods may also remove some true colour. Results after centrifugation may vary with the sample nature and size and speed of the centrifuge. When sample dilution is necessary it can alter the measured colour, if large colour-bodies are present. While reporting results,the pretreatment adopted need to be mentioned.

The visual comparison method is applicable to nearly all samples of potable water. Pollution by certain industrial waste may produce unusual colours that cannot be matched. In such cases an instrumental method is useful.

PLATINUM-COBALT METHOD :

Principle : Colour is determined by visual comparison of sample with known concentration of coloured solution Colour is expressed in terms of Hazan standard unit which is defined as the colour produced by 1 mg/l of platinum in the form of chloroplatinic acid in the presence of 2 mg/l of

cobaltous chloride hexahydrate. If it is not possible to match the colour with the standards the yellow component should be determined by the photoelectric method.

Interference :

1. Turbidity in excess of 5 units may seriously affect the colour of the samples.

2. Colour is extremely pH dependent and invariably increases as the pH of the sample increases. (pH should be determined at the same time as the colour).

Apparatus :

Nessler's tubes, matched 50 ml tall form, pH meter, spectrophotometer, Filteration system, Vacuum system.

Reagents :

1. **Stock standard colour solution :** Dissolve 1.246 gm K_2PtCl_6 (equivalent to 500 mg metallic platinum) and 1.00 gm crystallized $CoCl_2.6H_2O$ (equivalent to about 250 mg metallic cobalt). In distilled water that has been filtered to remove any slight turbidity add 100 ml conc.HCl and dilute with distilled water to 1 litre mark. This stock standard solution has a colour of 500 units.

2. **Working standard colour solution :** Prepare standards having colour of 5,10,15,20,25,30,35,40,45,50,60 and 70 by diluting 0.5,1.0,2.0, 2.5,3.0,4.5,5.0,6.0,7.0 ml in nessler tubes.

Procedure :

A. Field Method :

1. Since the platinum cobalt standard is not convenient for field, the colour of the sample may be compared with that of glass disks

held at the end of metallic tube containing glass comparator tubes of sample and colourless distilled water.

2. Calibrate every individual disk against standard colour on platinum cobalt scale.
3. Match the colour of the sample with the colour of the disk viewed by looking towards a white surface.
4. The method is recognised as a standard field procedure.

B. Laboratory Method : (Visual comparison)

1. Observe the colour of a sample by filling a matched tube to the 50 ml mark with the water to be examined.
2. Look vertically downward through the tube toward a white surface.
3. If turbidity is not removed, report the colour as apparent colour.
4. If coulur exceeds 70 units, dilute the sample with distilled water until the colour is within the range of standards.
5. Because the colour is related to pH measure the pH of each sample.

Calculation :

i) Calculate the colour units as follows :

Colour units : $\frac{A \times 50}{B}$

Where A = estimated colour of a dilute sample.

B = ml of a sample taken for dilution.

ii) Report the colour in whole number.

iii) Report the pH of the water sample.

2. Photoelectric method :

1. Filter the sample through a glass fibre filter paper to remove any turbidity.

2. Measure the O.D. (Optical Density) with a spectrophotometer at a wave-length of between 385 and 470 mm. or absorption meter with a suitable filter using 100 mm. cells with filtered water in reference cell.

3. From calibration graph (prepared in the range of 10 to 200 units) determine the colour of the sample.

4. Record as colour (instrumental) to the nearest whole number.

CONDUCTIVITY

Conductivity is a capacity of water to carry an electrical current and varies both with the number and types of ions the solution contains, which in turn is related to the concentration of ionized substances in the water. Most dissolved inorganic substances in water are in the ionised form and hence contribute to conductance. Rough estimation of dissolved ionic contents of water sample can be done by multiplying specific conductance (in μ siemens/cm) by an emperical factor which may vary from 0.55 to 0.9 depending on the soluble components of water and on the temperature of measurement. Conductivity measurement gives rapid and practical estimate of the variations in the dissolved mineral contents of a water supply.

Procedure :

Conductivity measurement is affected by :

1. The nature of the various ions, their relative concentration and the ionic strength of water.
2. Dissolved CO_2.
3. Turbidity
4. Temperature (For precise work, the conductivity must be determined at 25°C).

Most of the instruments commercially available for measurement of conductivity consists of.

i) A source of alternating current.

ii) A wheat-stone bridge, a null indicator, and,

iii) A conductivity cell consisting of a pair of rigidly mounted electrodes, each conductivity cell has its own cell constant : depending on its shape, size and the position of the electrodes. Either the cell constant is mentioned by the supplier or can be determined by using standard solution of KCl (0.01 M). Alternatively, by comparison with a cell of known cell constant. Other instruments measure the ratio of alternating current through the cell to voltage across it and have advantage of linear reading of

conductance. Portable battery operated instruments for both pH and conductivity are also available for field studies.

Conductivity can be measured as per the instruction manual supplied with the instrument and the results may be expressed as siemens/metre **or** μ siemens/cm at temperature say 25°C at which measurement was made. With reasonable care conductivity meter needs very little maintenance and gives accurate results. However few important points in this respect are :

1. Adherent coating formation of the sample substances on the electrodes should be avoided which requires thorough washing of cell with distilled water at the end of each measurement.

2. Keep the electrode immersed in distilled water

3. Organic material coating can be removed with alcohol or acetone followed by washing with distilled water.

TURBIDITY :

Suspension of particles in water interfering with passage of light is called turbidity. Turbidity is caused by wide variety of suspended matter which range in size from colloidal to coarse dispersions depending upon the degree of turbulance and also ranges from pure in-organic substances to those that are highly organic in nature. Turbid waters are undesirable from aesthetic point of view in drinking water supplies and may also affect products in industries. Turbidity is measured to evaluate the performance of water Treatment plant.

Principle : Turbidity can be measured either by its effect on the transmission of light which is termed as Turbidimetry or by its effect on the scattering of light which is termed as Nephelometry. Turbidometer can be used for sample with moderate turbidity and nephelometer for samples with low turbidity. Higher the intensity of scattered light higher the turbidity.

Interference : Colour is the main source of interference in the measurement of turbidity.

Apparatus : Turbidimeter or Nephelometer.

Reagents :

1. Solution I : Dissolve 1.0 gm Hydrazine sulphate and dilute to 100 ml.

2. Solution II: Dissolve 10.0 gm hexamethylene tetramine and dilute to 100 ml.

3. Mix 5 ml of I with 5 ml of II. Allow to stand for 24 hrs. at 25 $\pm$ 3°C and dilute to 100 ml. This solution (III) will have turbidity of 400 units (N.T.U.)

4. Standard turbidity suspension : Dilute 10 ml of solution III as prepared above to 100 ml to have solution of the turbidity of 40 units. (N.T.U.)

Procedure :

1. Prepare calibration curve in the range of 0-400 units by carrying out appropriate dilutions of solutions III and IV above taking readings on turbidimeter.

2. Take sample or a suitably diluted aliquote and determine its turbidity either by visual comparison with the diluted standards or by reading on turbidimeter.

3. Read turbidity from the standard curves and apply correction due to dilution, if necessary.

4. Report the readings in turbidity units.

TOTAL DISSOLVED (FILTERABLE) SOLIDS

The dissolved (Filterable) solids can be determined from the difference between the residue on evaporation and total suspended solids, but if the dissolved solids content is low and the suspended solids high, a direct determination is better. It is preferable to adopt the centrifugal method of separating suspended matter in order that a sufficiently large volume of seperated liquid is available for the determination.

Principle : A known volume of filtered sample is evaporated and dried in a weighed dish at 105°C to constant weight the increase in weight over the empty dish represents the dissolved solids.

Apparatus :

1. Evaporating dishes, 50,100 ml capacity (Preferable porcelain or silica).
2. Pipettes 25,50 ml capacity
3. Water bath & Oven
4. Balance to weigh up to 4th decimal.

Procedure :

The known volume (V) of filtered sample in a previously ignited and weighed basin (W_1). Evaporate to dryness on a steam bath and further dry at 105°C for one or two hours in an oven. Cool in dessicator and weigh (W_2). Repeat by further heating for 15 minutes and cooling until successive results do not differ by more than about 0.4 mg.

Calculation :

$$\text{Dissolved solids mg/l} = \frac{(W_2 - W_1) \times 1000}{V}$$

pH :

For most practical purposes the pH of aqueous solutions can be taken as negative logarithm of hydrogen ion activity. pH values from 0 to 7 are diminishingly acidic, 7 to 14 increasingly alkaline and 7 is neutral.

The pH of natural water usually lies in the range of 4.4 to 8.5. Its value is governed largely by the carbon dioxide/bicarbonate/carbonate equilibrium. It may be affected by humic substances by changes in the carbonate equilibria due to the bioactivity of plants and in some cases by hydrolyzable salts. The effect of pH on the chemical and biological properties of liquids makes its determination very important. It is used in several calculations in analytical work and its adjustment is necessary for some analytical procedures.

The determination of pH by conventional chemical means is not practicable and the equilibria which are involved depend to some extent on temperature. The precise accepted scale of pH must therefore be based on an agreed primary standard. The colorimetric indicator methods can be used only if approximate pH values are required.

The pH determination is usually done by electrometric method which is the most accurate method and free of interferences.

ELECTROMETRIC METHOD :

The pH is determined by measurement of the electromotive force of a cell comprising an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and a reference electrode (usually a mercury calomel electrode) contact between the test solution and the reference electrode is usually achieved by means of a liquid junction, which forms a part of the reference electrode. The emf of this cell is measured with pH meter. This is a high impedance electrometer calibrated in terms of pH.

Apparatus :

Glass Electrode This must be compatible with the pH meter used and must be suitable for the particular application. Special electrodes are available for pH values greater than 10 and for use at temperature greater than 60°C. Combined glass/reference electrodes are also available and are convenient to use.

Reference Electrode : The mercury/calomel electrode is widely used but the silver/silver chloride electrode may be preferable on account of it being more reproducible and more reliable. Less concentrated solutions of KCl(e.g. 3.5 M KCl or 350 gm/litre) are more satisfactory as filling solutions than the saturated solution often used because problems due to clogging of the electrode or the liquid junction will be avoided. To prevent dissolution of the silver chloride film the potassium chloride filling solution of Ag/Agcl electrodes should be saturated with AgCl.

pH Meter : Both mains and battery operated models are available, the later type can be used for fields measurements. The most accurate pH meters can be read to better than ± 0.005 pH unit.

Reagents :

1. **Buffer solution for pH 4.0 :** Dissolve 10.12 gm potassium dihydrogen phthalate dried at 110°C in freshly distilled water and dilute to one litre at 25°C.
2. **Buffer solution for pH 6.8 :** Dissolve 3.388 gm anhydrous KH_2PO_4 and 3.533 gm Na_2HPO_4 both of which have been dried overnight at between 110°C and 130°C in water and dilute to 1 litre at 25°C. The distilled water should be freshly boiled, cooled and free from CO_2.
3. **Buffer solution for pH 9.2 :** Dissolve 3.80 gm $Na_2B_4O_7 . 10\ H_2O$ in water dilute to 1 litre at 25°C.

NOTE : In general analytical reagent grade chemicals are satisfactory for the preparation of these solutions. Commercial buffer tablets are available in the market for the preparation of solution of above pH

values (each tablet dissolved in 100 ml gives the buffer solution of required pH).

Procedure :

1. Standardize the pH meter according to the manufacturer's instructions.
2. Select a standard buffer solution with a pH value close to that of the water to be treated.
3. Set the temperature control to the temperature of the buffer.
4. Set the meter to the pH of the buffer at that temperature.
5. Check the electrode response by measuring a second standard buffer solution of different pH.
6. Wash the elctrode thoroughly first with distilled water and then with the sample.
7. Set the temperature control to the temperature of the sample.
8. Immerse electrodes in the sample and record the pH after stabilising the system.

NOTE : Between measurements, the electrodes are kept in distilled water. New or dried out glass electrodes should be prepared for the use by soaking in 0.1 N HCl for 8 hours or according to the maker's instructions.

ALKALINITY :

The alkalinity of water is a measure of its capacity to neutralise acids. The alkalinity of natural water is due to the salts of carbonate, bicarbonate, borates, silicates and phosphates along with the hydroxyl ions in free state. However the major portion of the alkalinity in natural waters is caused by hydroxide, carbonate and Bicarbonates which may be ranked in order of their association with high pH values. Alkalinity values provide guidance in applying proper doses of chemicals in water and waste water treatment processes, particularly in coagulation and softening.

Principle : Alkalinity of a sample can be estimated by titrating with standard sulphuric acid. Titration to pH 8.3 or decolourisation of phenolphthalein indicator will indicate complete neutralisation of pH and 1/2 of CO_3 while to pH 4.5 or sharp change from yellow to pink of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH, CO_3, HCO_3).

Reagents :

1. **Standard H_2SO_4 (0.02 N)** : Prepare 0.1 N H_2SO_4 by diluting 3.0 ml conc. H_2SO_4 to 1000 ml. Standardise it against standard Na_2CO_3 0.1 N. Dilute appropriate volume of H_2SO_4 (approx. 0.1 N) to 1000 ml to obtain standard 0.02 N H_2SO_4.

2. **Phenolphthalein indicator** : Dissolve 5 gm in 500 ml 95% ethyl alcohol. Add 500 ml distilled water. Add dropwise 0.02 N NaOH till faint pink colour appears.

3. **Methyl orange indicator** : Dissolve 0.5 gm and dilute to 1000 ml with CO_2 free distilled water.

Procedure :

1. Take 25 or 50 ml sample in a conical flask and add 2-3 drops of phenolphthalein indicator.

2. If pink colour develops titrate with 0.02 N H_2SO_4 till it disappears or pH is 8.3 Note the volume of H_2SO_4 required.

3. Add 2-3 drops methyl orange to the same flask,and continue titration till pH down to 4-5 or orange colour changes to pink.

Note the vol. of H_2SO_4 added ..... B

4. In case pink colour does not appear after addition or phenolphthalein continue as in 3 above.

5. Calculate Total (T) phenolphthalein (P) and methyl orange alkalinity as follows and express in mg/l as $CaCO_3$.

   P - Alkalinity, mg/l as $CaCO_3$ = A x 1000/ml sample.

   MO - Alkalinity, mg/l as $CaCO_3$ = B x 1000/ml sample.

   T - alkalinity, mg/l as $CaCO_3$ = (A+B) x 1000/ml sample

   In case H_2SO_4 is not 0.02 N apply the following formula:

   $$\text{Alkalinity, mg/l as } CaCO_3 = \frac{A \times N \times 50000}{\text{ml Sample}}$$

   Where N = Normality of H_2SO_4 used.

Once the phenolphthalein and total alkalinity is determined, then three types of alkalinities i.e. hydroxide, carbonate and bicarbonate are easily, calculated from the table given below.

Values of P & T	**OH**	**CO_3**	**HCO_3**
P = 0	0	0	T
P < 1/2 T	0	2p	T-2p
P = 1/2 T	0	2p	0
P > 1/2 T	2p-T	2(T-P)	0
P = T	T	0	0

CHLORIDE

Chloride ion is generally present in natural waters. The presence of chloride in natural waters can be attributed to dissolution of salt deposits, discharges of effluents from chemical industries, irrigation drainage, contamination from refuge leachates and sea water intrusion in coastal areas. The salty taste produced by chloride depends on the chemical composition of the water. A concentration of 250 mg/l may be detectable in some waters containing sodium ions. A high chloride content has a deleterious effect on metallic pipes and structures as well as on agricultural plants.

Principle : Chloride is determined in a neutral or slightly alkaline solution by titration with standard silver nitrate, using potassium chromate as an indicator silver chloride is quantitatively precipitated before red silver chromate is formed.

Interference : If the sample is too coloured or turbid to allow the end point to be readily detected, this interference may be reduced by alum flocculating followed by filtration prior to the estimation of chloride.

Reagents :

1. **Potassium chromate indicator :** Dissolve 50 g K_2CrO_4 in distilled water. Add $AgNO_3$ till definite red precipitate is formed. Allow to stand for 12 hrs. Filter and dilute to 1000 ml.
2. **Silver nitrate (0.0141 N) :** Dissolve 2.395 gm $AgNO_3$ and dilute to 1000 ml. Standardize against NaCl, 0.141 N. 1 ml of 0.141 N $AgNO_3$ = 0.5 mg Cl.
3. **Sodium chloride 0.141 N :** Dissolve 824.1 mg NaCl Dried at 140°C) and dilute to 1000 ml. 1 ml = 0.5 mg Cl.
4. **Special reagent to remove colour and turbidity:** Dissolve 125 g AlK $(SO_4)_2$, 12 H_2O or $AlNH_4(SO_4)_2 \cdot 12H_2O$ and dilute to 1000 ml Warm to 60°C and add 55 ml conc. NH_4OH slowly. Allow to stand for 1 hr. Solution should be free from Cl.

Procedure :

1. Take 100 ml sample and adjust the pH between 7.0 and 8.0
2. Take 50 ml well mixed sample adjusted to pH 7.0 - 8.0 and add 1.0 ml K_2CrO_4
3. Titrate with standard $AgNO_3$ solution till $AgCrO_4$ starts precipitating
4. Standardize $AgNO_3$ against standard NaCl.
5. For better accuracy titrate distilled water (50 ml) in the same way to establish reagent blank
6. Calculate as follows :

$$\text{Chloride mg/l} = \frac{(A-B) \times N \times 35.45 \times 1000}{\text{ml sample}}$$

Where A = ml $AgNO_3$ required for sample.

B = " " " blank.

N = Normality of $AgNO_3$ used.

HARDNESS

Water hardness is the traditional measure of the capacity of water to react with soap, hard water requiring a considerable amount of soap to produce a lather. Hardness of water is not a specific constituent but a variable and complex mixture of cations and anions. The principle hardness causing ions are calcium and magnesium. The degree of hardness of drinking water has been classified in terms of the equivalent $CaCO_3$ concentration as follows

Soft 0-60 mg/l
Medium 60-120 mg/l
Hard 120-180 mg/l
Very hard > 180 mg/l

Hardness many also be discussed in terms of carbonate (Temporary) and Non-carbonate (permanent) Hardness. Carbonate hardness can be removed or precipitated by boiling. This type of hardness is responsible for the deposition of scale in hot water pipes and kettles. Non-carbonate hardness is caused by the association of the hardness causing cations with sulphate,chloride or nitrate. It cannot be removed by boiling. Public acceptability of the degree of hardness may vary considerably from community to community, depending on local conditions.

EDTA TITRIMETRIC METHOD :

Principle : In alkaline condition EDTA reacts with Ca and Mg to form a soluble chelated complex. Ca and Mg ions develop wine red colour with Eriochrome Black - T under alkaline condition. When EDTA is added as a titrant the Ca and Mg divalent ions get complexed resulting in sharp change from wine red to blue which indicates end point of the titration. The pH for this titration has to be maintained at 10.0 ± 0.1. At a high pH i.e.about 12.0 Mg ion precipitates and only Ca^{++} ion remains in solution. At this pH Murexide indicator forms a pink colour with Ca^{++}. When EDTA is added Ca^{++} gets complexed resulting in a change of colour from pink to purple which indicates the end point of the reaction.

Intereference : Metal ions do intefere but can be overcome by addition of inhibitors.

Reagents

1. **Buffer solution :** Dissolve 16.9 gm. NH_4Cl in 143 ml NH_4OH. Add 1.25 gm. magnesium salt of EDTA to obtain sharp change in indicator and dilute to 250 ml. If magnesium salt of EDTA is unavailable, dissolve 1.179 gm. disodium salt of EDTA (AR grade) and 780 mg $MgSO_4.7H_2O$ or 644 mg $MgCl_2.6H_2O$ in 50 ml distilled water. Add to above solution of NH_4Cl in NH_4OH and dilute to 250 ml.

2. **Inhibitor :** Dissolve 4.5 gm hydroxyl-amine hydrochloride in 100 ml 95% ethyl alcohol or isopropyl-alcohol.

3. **Eriochrome Black T indicator :** Mix 0.5 gm. dye with 100 gm. Nacl to prepare dry powder.

4. **Murexide Indicator :** Prepare a ground mixture of 200 mg of murexide (ammonium purpurate) with 100 gm of solid NaCl.

5. **Sodium hydroxide 2 N :** Dissolve 80 gm NaOH and dilute to 1000 ml.

6. **Standard EDTA soultion 0.01 M :** Dissolve 3.723 gm EDTA sodium salt and dilute to 1000 ml. Standardize against standard calcium solution, 1 ml = 1 mg $CaCO_3$.

7. **Standard calcium solution :** Weigh accurately 1.0 gm AR grade $CaCO_3$ and transfer to 250 ml conical flask. Place a funnel in the neck of a flask and add 1+1 HCl till $CaCO_3$ dissolves completely. Add 200 ml distilled water and boil for 20-30 min. to expel CO_2. Cool and add methyl red indicator. Add NH_4OH 3 N dropwise till intermediate orange colour develops. Dilute to 1000 ml to obtain 1 ml = 1 mg $CaCO_3$.

Procedure :

A total Hardness :

1. Take 25 or 50 ml well mixed sample in porcelain dish or conical flask.

2. Add 1-2 ml buffer solution followed by 1 ml inhibitor.

3. Add a pinch of Eriochrome Black T and titrate with standard EDTA (0.01 M) till wine red colour changes to blue. Note down the volume of EDTA required (A)

4. Run a reagent blank. Note the volume of EDTA .....(B)

5. Calculate volume of EDTA required by sample, from volume of EDTA required in steps 3 & 4

$$C = (A - B)$$

6. Calculate as follows :

   Total hardness

   $$\text{as } CaCO_3 \quad mg/l = \frac{C \times D \times 1000}{\text{ml sample}}$$

   Where C = Vol. of EDTA required by sample.

   D = mg $CaCO_3$ per 1.0 ml. EDTA (0.01 M) used as titrant

B. Calcium Hardness :

1. Take 25 or 50 ml sample in porcelain dish.
2. Add 1 ml NaOH to raise pH to 12.0 and a pinch of murexide indicator.
3. Titrate immediately with EDTA till pink colour changes to purple. Note the vol. of EDTA used (A').
4. Run a reagent blank. Note the ml. of EDTA (B') required and keep it aside to compare end points of sample titrations.
5. Calculate as follows :

   $$\text{Calcium hardness as } CaCO_3 = \frac{C' \times D \times 1000}{\text{ml sample.}}$$

Where C' = volume of EDTA used by sample (A'-B')

D = mg $CaCO_3$ per 1.0 ml EDTA (0.01 M) used for titration.

C. **Magnesium hardness** as $CaCO_3$ mg/l = total hardness as $CaCO_3$ mg/l - Ca hardness as $CaCO_3$ mg/l

D. **Alkaline (Carbonate) Hardness and Non Alkaline (Non Carbonate) Hardness.**

These types of hardness can be calculated from total hardness and total alkalinity data as follows :

If total hardness as $CaCO_3$ $>$ Total alkalinity as $CaCO_3$

Then

1. Alkaline Hardness = Total alkalinity.
2. Non-alkaline Hardness = Total hardness - total alkalinity

If total hardness as $CaCO_3$ $<$ Total alkalinity as $CaCO_3$

Then,

I) Alkaline hardness = Total hardness

II) Non alkaline hardness = Nil.

SULPHATE

Sulphate ions usually occur in natural water. Many sulphate compounds are readily soluble in water. Most of them originated from the oxidation of sulphite ores, the solution of gypsum and anhydrite, the presence of shales, particularly those rich in organic compounds. Atmospheric sulphurdioxide formed by the combustion of fossil fuels and emitted by the metallurgical roasting processes may also contribute to the sulphate compounds of water. Sulphate bearing Minerals are common in most sedimentary rocks. In the weathering process gypsum ($CaSO_4$) is dissolved and sulphide minerals are partly oxidised, giving rise to a soluble form of sulphate that is carried away by water. Sulphates cause a problem of scaling in industrial water supplies, and problems of odor and corrosion in waste water treatment due its reduction to H_2S. Ingestion of water containing high concentration of sulphate can have a laxative effect, which is enhanced when sulphate is consumed in combination with magnesium.

Turbidimetric Method

Principle : Sulphate ions are precipitated as $BaSO_4$ in acidic media (HCL) with Barium chloride. The absorption of light by this precipitated suspension is measured by spectrophotometer at 420 nm or scattered light by Nephelometer.

Interference : Colour, turbidity and silica in the concentration of 500 ppm interfere in this estimation. Filtration is adopted to remove colour and turbidity.

Apparatus :

1. Magnetic stirrer
2. Colorimeter for use at 420 nm
3. Stopwatch
4. Nessler's tubes, cap 100 ml.
5. Measuring spoon cap - 0.2 to 0.3 ml.

Reagents :

1. **Conditioning reagent :** Mix 50 ml. glycerol with a solution containing 30 ml. concentrated HCL, 300 ml. distilled water, 100 ml. 95% ethyl or isopropyl alcohol and 75 g NaCL.
2. **Barium chloride.** Crystals. 20-30 mesh.
3. **Standard sulphate solution :** Dissolve 147.9 mg. anhydrous Na_2SO_4 and dilute to 1000 ml.
   1 ml. = 100 ug SO_4.

Procedure

1. Take suitable vol. of sample in 250 ml. conical flask and dilute to 100 ml.
2. Add 5.0 ml. conditioning reagent accurately. Mix well.
3. Keep the flask constantly stirred with the help of stirrer. Add $BaCl_2$ crystals while stirring. Continue stirring for 1 min. after addition of $BaCl_2$.
4. Measure the turbidity developed after every 30 sec. for 4 min. on colorimeter at 420 nm. After 2 min. Stirring, reading will remain constant. Note this reading for calculation purpose.
5. Prepare standard curve by carrying standard sulphate solution through entire procedure. Space standards at 5 mg/l increment in the 0 to 40 mg/l range.
6. Read mg SO_4 present in the sample from the standard curve.
7. Calculate as follows :

$$mg/l\ SO_4 = \frac{mg\ SO_4 \times 1000}{ml\ Sample}$$

FLUORIDES :

Fluoride ions have dual significance in water supplies. High concentration of F^- causes dental fluorisis (Disfigurement of the teeth). At the same time, a concentration less than 0.8 mg/l results in 'dental caries'. Hence it is essential to maintain the F^-conc. between 0.8 to 1.0 mg/l in drinking water. Among the many methods suggested for the determination of fluoride ion in water, the colorimetric method (SPADNS) & 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 while addition of the prescribed buffer frees the electrode method from the interference caused by such relatively common ions as aluminium, hexametaphosphate, and orthophosphate which adversely affect the colorimetic methods. However samples containing fluoroborate ion (BF_4) must be subject to preliminary distillation step in either of the methods. Both the methods and the preliminary distillation step are discussed below.

1. SPADNS METHOD :

Principle : Under acid condition fluorides (HF) react with zirconium SPADNS solution and the 'Lake' (colour of SPADNS reagent) gets bleached due to formation of ZrF_6 . Since bleaching is a function of fluoride ions, it is directly proportional to the concn. of F . It obeys Beer's law in a reverse manner.

Interference : Alkalinity 5000 mg/l, aluminium 0.1 mg/l, chlorides 7000 mg/l, Fe 10 mg/l, PO_4 16 mg/l, $SO_4$200 mg/l, and hexametaphosphate 1.0 mg/l interfere in the bleaching action. In presence of interfering radicals distillation of sample is recommended.

Apparatus

1. Distillation apparatus (as shown in the Fig. 3)
2. Colorimeter for use at 570 nm.
3. Nessler's tubes cap. 100 ml.

Reagents

1. Sulphuric acid H_2SO_4 conc.
2. Silver sulphate Ag_2SO_4 crystals.
3. SPADNS solution : Dissolve 958 mg SPADNS and dilute to 500 ml.
4. Zirconyl acid reagent : Dissolve 133 mg $ZrOCl_2.8H_2O$ in 25 ml water. Add 350 ml conc. HCl and dilute to 500 ml.
5. Mix equal volume of 3 and 4 to produce a single reagent. Protect from direct light.
6. Reference solution : Add 10 ml SPADNS solution to 100 ml distilled water. Dilute 7 ml conc. HCl to 10 ml and add to diluted SPADNS solution.
7. Sodium arsenite solution : Dissolve 5.0 g $NaAsO_2$ and dilute to 1000 ml.
8. Stock F^- solution : Dissolve 221.0 mg an hydrous NaF and dilute to 1000 ml. 1 ml = 100 μg F^-.
9. Standard F : Dilute stock solution 10 times to obtain 1 ml = 10 μg F^-.

A. Preliminary Distillation Step

Place 400 ml distilled water in the distilling flask and carefully add 200 ml conc. H_2SO_4. Swirl until the flask contents are homogenous, add 25 to 30 glass beads and connect the apparatus as shown in fig 3. Begin heating slowly at first and then rapidly until the temperature of the flask reaches exactly 180°C. Discard the distillate. This process removes fluoride contamination and adjusts the acid-water ratio for subsequent distillations.

After cooling, the acid mixture remaining after above step or previous distillation to 120°C or below add 300 ml of sample, mix thoroughly, and distill as before until the temperature reaches 180°C. Do not heat above 180°C to prevent sulphate carryover.

Add Ag_2SO_4 to distilling flask at the rate of 5 mg/mg Cl when high chloride samples are distilled. Use the sulphuric acid solution in the flask repeatedly until the contaminants from the samples

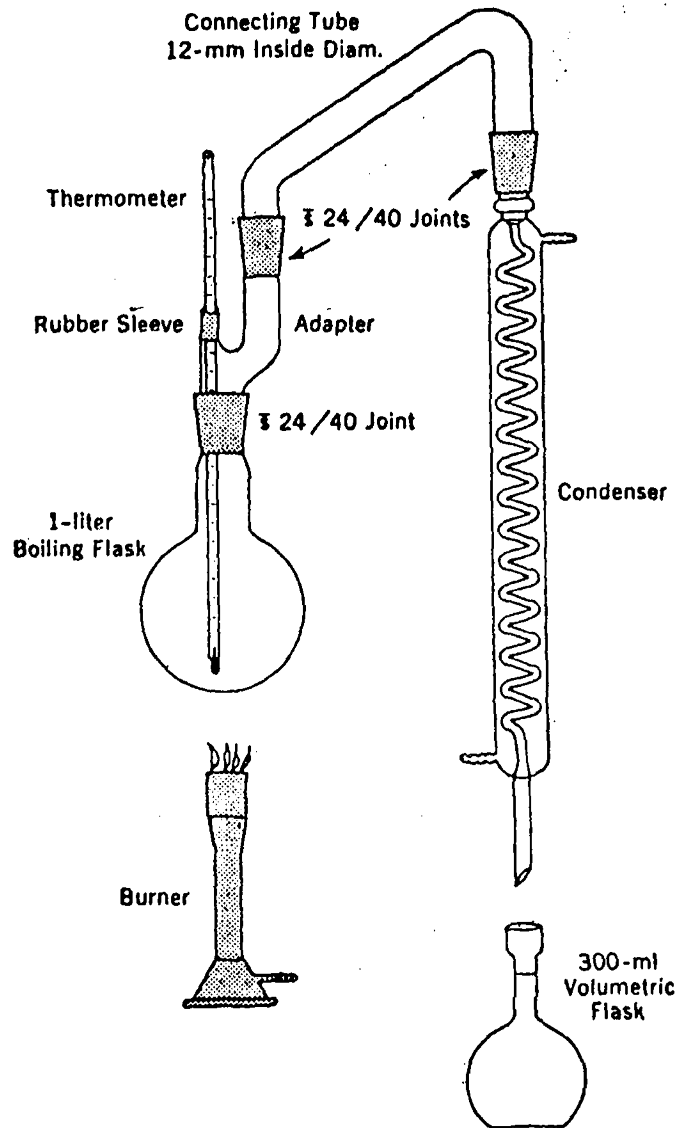


Fig.3: Direct Distillation Assembly for Fluoride

accumulate to such an extent that recovery is affected or interferences appear in the distillate. After the distillation of high fluoride samples, flush the still with 300 ml distilled water and combine the two fluoride distillates. After Periods of inactivity, similarly flush the still, discard the distillate.

B. Procedure. :

1. Prepare standard curve in the range 0.0 to 1.40 mg/l by diluting appropriate volume of standard F solution to 50 ml in Nessler's tubes.
2. Add 10.0 ml mixed reagent prepared as in 5 above to all the samples, mix well and read optical density of bleached colour at 570 nm using reference solution for setting zero absorbance.
3. Plot concn. Vs % transmission or absorbance.
4. If sample contains residual chlorine remove it by adding 1 drop (0.05ml) $NaAsO_2$ solution 0.1 mg Cl_2 and mix. $NaAsO_2$ concn. should not exceed 1300 mg/l to avoid error due to $NaAsO_2$. Take suitable aliquot & dilute it to 50 ml.
5. Add acid Zirconyl - SPADNS reagent 10 ml; Mix well and read % transmission or absorbance.
6. Take suitable aliquots of sample either direct or after distillation in Nessler's tubes Follow the step 5.
7. Calculate the mg F present in the sample using standard curve.

2. ION SELECTIVE ELECTRODE METHOD :

Principle : The fluoride sensitive electrode is of the solid state type, consisting of a lanthanum fluoride crystal; in use it forms a cell in combination with a reference electrode, normally the calomel electrode. The crystal contacts the sample solution at one face and an internal reference solution at the other. A potential is established by the presence of fluoride ions across the crystal which is measured by a device called ion 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 bound or complexed fluoride. Addition of a buffer solution of high total ionic strength containing a chelate to complex aluminium preferentially overcomes these difficulties.

Interference : Polyvalent cations such as Al(III), Fe(III) and Si(IV) will complex fluoride ions. However the addition of CDTA (Cyclohexylene diamine tetra acetic acid) preferentially wil complex concentrations of aluminium up to 5 mg/L. Hydrogen ion forms complex with fluoride while hydroxide ion interferes with electrode response. By adusting the pH in between 5 to 8 no interference occurs.

Apparatus :

1) Ion meter (field/laboratory model) or pH/mV meter for precision laboratory measurements.
2) Reference electrode (calomel electrode)
3) Fluoride sensitive electrode.
4) Magnetic stirrer.
5) Plastic labwares (Samples and standards should always be stored in plastic containers as fluoride reacts with glass).

Reagents :

1) Standard fluoride solution prepared as directed in SPADNS method.
2) Total Ionic strength adjustment buffer (TISAB)

Place approximately 500 ml distilled water in a 1 - L beaker add 57 ml glacial acetic acid, 58 gm NaCl and 4.0 gm 1,2 cyclohexylene diamine tetraacetic acid. Stir to dissolve. Place beaker in a cool water bath and add slowly 6 N NaOH (About 125 ml) with stirring, until pH is between 5 and 5.5. Transfer to a 1 - L volumetric flask and make up the volume to the mark.

Procedure :

1) For connecting the electrodes to meter, and for further operation of the instrument follow the instruction manual supplied by the manufacturer.
2) Check the electrode slope with the ionmeter (59.16 mV for monovalent ions and 29.58 mV for divalent ions at 25°C)
3) Take 50 ml of each 1 ppm and 10 ppm fluoride standard. Add 50 ml TISAB (or 5 ml if conc. TISAB is used) and calibrate the instrument.
4) Transfer 50 to 100 ml of sample to a 150 ml plastic beaker. Add TISAB as mentioned in (3).
5) Rinse electrode, blot dry and place in the sample. Stir throughtly and note down the steady reading on the meter.
6) Recalibrate every 1 or 2 hours.
7) 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.
8) 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. From the change in electrode potential before and after addition, the original sample concentration is determined.

NITRATE

Nitrate is the most highly oxidized form of nitrogen compounds commonly present in natural waters, because it is the product of the aerobic decomposition of organic nitrogenous matter. Significant sources of nitrate are chemical fertilizers., decayed vegetable and animal matter, domestic effluents sewage sludge disposal to land, industrial discharge, leachates from refuse dumps and atmospheric washout. Depending on the situation, these sources can contaminate streams, rivers, lakes and ground water, especially wells. Unpolluted natural water contain usually only minute amount of nitrate. Excessive concentration in drinking water are considered hazardous for infants because in their intestinal track nitrates are reduced to nitrites which may cause 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 objectional eutrophication .

Conventional method of PDA and ultraviolet spectrophotometric method especially used for those samples that have low organic matter contents, that is unpolluted natural waters, are the two methods of nitrate estimation which are discussed below :

PHENOL DISULPHONIC ACID (PDA) METHOD

Principle : Nitrate reacts with phenol disulphonic acid and produces a nitro-derivative which in alkaline solution developes yellow colour due to rearrangement of its structure. The colour produced follows Beer's law and is proportional to the concentration of NO_3 present in the sample.

Interference : Chlorides and nitrate, are the two main sources of interference. Pretreatment of sample is necessary when the interfering radicals are present.

Apparatus :

1. Colorimeter or spectrophotometer having a range of 300-700 nm.

2. Nessler's tubes Capacity 100 ml.
3. Beakers Capacity 100 ml.
4. Water bath Capacity 100 ml.

Reagents :

1) **Standard silver sulfate:** Dissolve 4.40 g Ag_2SO_4 in distilled water and dilute to 1000 ml, 1 ml = 1 mg Cl.
2) **Phenol disulfonic acid(PDA) :** Dissolve 25 g white phenol in 150 ml conc. H_2SO_4 Add 75 ml fuming H_2SO_4 (15% free SO_3) stir well and heat for 2 hr. on waterbath. If fuming sulfuric acid is not available add additional 85 ml conc. H_2SO_4 to the 150 ml $H2SO_4$ stir well and heat for 2 hr.
3. Ammonium hydroxide concentrated.
4. **Potassium hydroxide 12 N :** Dissolve 673 g KOH in distilled water and make up to 1000 ml with distilled water for hard waters use liquid ammonia.
5. **Stock nitrate solution :** Dissolve 721.8 mg anhydrous potassium nitrate and dilute to 1000 ml with distilled water.

   1 ml = 100 μ g N.
6. **Standard nitrate solution :** Evaporate 50 ml stock NO_3 to dryness on water bath. Dissolve residue in 2 ml PDA reagent and dilute to 500ml.

   1 ml = 10 μ g N.
7. **EDTA :** Rub 50 g EDTA with 20 ml distilled water to form a paste. Add 60 ml NH_4OH and mix well.
8. **Aluminium hydroxide :** Dissolve 125 g potash alum in 1000 ml distilled water. Warm to 60°C and add 55-60 ml NH_4OH and allowed to stand for 1 hr. Decant the supernatant and wash the precipitate a number of times till it is free from Cl, NO_2 and NO_3. Finally after settling, decant off as much clean liquid as possible, leaving only the concentrated suspension.

Procedure :

a) Pretreatment of sample

1. **Colour removal :** If the sample has a colour in excess of 10 units;

add 3 ml aluminium hydroxide to 150 ml sample. Stir well and allow to settle for a few min. Filter and use the filtrate discarding the first portion of the filtrate.

2. **Nitrite removal :** (1) Generally NO_2 occurs along with NH_3 and gets eliminated in the routine test due to decomposition of NO_2 and NH_3 to N_2, (ii) Oxidize NO_2 to NO_3 under acid condition using $KMnO_4$, (iii) Add sulfamic acid to the sample to suppress NO_2 interference.
3. **Chloride removal :** Determine the Cl contents of the sample and precipitate out as AgCl. One should be vėry careful while adding $Ag2SO_4$ because excess Ag will precipitate out as silver oxide when alkali is added to sample.

b) Colour Development

1. Neutralize the clarified sample to pH 7.0.
2. Take suitable aliquot of the sample in a beaker and evaporate to dryness on waterbath.
3. Dissolve the residue using glass rod with 2 ml phenol disulfonic acid reagent. Dilute and transfer to Nessler tubes.
4. Add 8-10 ml 12 N KOH. If turbidity is developed add the EDTA reagent dropwise till it dissolves. Filter and make up to 100 ml. To avoid this add 10 ml conc. NH_4OH instead of KOH.
5. Prepare blank in the same way using distilled water instead of sample.
6. Read the colour developed at 410 nm with a light path of 1 cm. Record NO_3 as N in mg/l.
7. Prepare calibration curve using suitable aliquots of standard NO_3 in the range of 5 to 500 μ g N/l following the above procedure.

2. UV Spectrophotometric Method :

Principle : Measurement of the ultraviolet absorption at 220 nm enables rapid determination of nitrate. The nitrate calibration curve follows Beer's law up to 11 mg/l N. Because dissolved organic matter may also absorb at 320 nm and nitrate does not absorb at 275 nm a second measurement can be made at 275 nm to correct the nitrate

value. The extent of this empirical correction is related to the nature and concentration of the organic matter 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.

Acidification with 1 N hydrochloric acid is designed to prevent interference from hydroxide or carbonate concentrations up to 1,000 mg/l as $CaCO_3$. Chloride has no affect on the determination. Minimum detectable concentration is 40 µ g/l nitrate N.

Interference : Dissolved organic matter, nitrate, hexavalent chromium, and surfactants interfere. The latter three substances may be compensated for by the preparation of individual correction curves.

Organic matter can cause a positive but variable interference, the degree depending on the nature and concentration of the organic material.

Note : Clean all glassware thoroughly and rinse to reduce the error that might result from streaks or particles on the outside of the cuvettes, 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 minimize colour interference.

Apparatus

1. Spectrophotometer, for use at 220 nm and 275 nm with matched silica cells of 1 cm or longer light path.
2. **Filter :** One of the following is required.
   i) Membrane filter : 0.45 nm membrane filter, and appropriate filter assembly.
   ii) Paper : Acid-washed, ashless hard-finish filter paper sufficiently retentive for fine precipitates.
3. Nessler tubes, 50 ml, short form.

Reagents

1. **Redistilled water :** Use redistilled water for the preparation of all solutions and dilutions.

2. **Stock nitrate solution :** Prepare as described in PDA method 1.00 ml = 100 μg N=443 μg NO_3.

3. **Standard nitrate solution :** Dilute 100.0 ml with stock nitrate solution to 1000 ml with distilled water : 1.00 ml = 10.0 μ g N = 44.3μ g NO_3.

4. Hydrochloric acid solution, HCl, 1.N.
5. **Aluminum hydroxide suspension :** Prepare as directed in PDA Method.

Procedure

a. **Colour removal :** If the sample has a high colour or is known to contain organic interference, add 4 ml $Al(OH)_3$ suspension/100 ml sample in an erlenmeyer flask. Swirl to mix and let settle for 5 min. Filter through a 0.45 μ m membrane filter previously washed with about 200 ml distilled water.

b. **Treatment of sample :** To 50 ml clear sample, filtered if necessary, or to 50 ml sample filtered after colour removal, add 1 ml 1N, HCl and mix thoroughly.

c. **Preparation of standard curve :** Prepare nitrate calibration standards in the range 0 to 350 μ g N by diluting to 50 ml the following volumes of the standard nitrate solution : 0.1.00, 2.00, 4.00, 7.00 ....35.0 ml. Treat the nitrate standards in the same manner as the samples.

d. **Spectrophotometric measurement :** 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 275 nm to obtain the interference due to dissolved organic matter.

Calculation

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

Calculate as follows :

$$\text{Nitrate N mg/l} = \frac{\text{Net } \mu\text{g nitrate N}}{\text{ml of Sample.}}$$

NO_3 mg/l = Nitrate N mg/l x 4.43.

DISSOLVED OXYGEN

All living organisms are dependent upon oxygen in one from or the other to maintain the metabolic processes that produce energy for growth and reproduction. Water which is in contact with oxygen or with an oxygen containing mixture of gases, contains some dissolved oxygen. When water is saturated by oxygen, the concentration of oxygen is called the equilibrium concentration. The real concentration of oxygen may differ from the equilibrium concentration because of physical, chemical and biochemical conditions or activities. It is poorly soluble gas and its solubility directly varies with the atmosphere pressure at any given temperature.

Analysis of DO is a key test in sanitary engineering practices. The following illustrations reveal the importance of DO as a parameter :

1. It is necessary to know DO levels to assess quality of raw water and to keep a check on stream pollution.
2. In liquid waste dissolved oxygen is the factor that determines whether the biological changes are fought out by aerobic or anearobic organisms.
3. DO test is the basis of BOD test which is an important parameter to evaluate pollution potential of wastes.
4. DO is necessary for all aerobic biological waste water treatment processes.
5. Oxygen is an important factor in corrosion. DO test is used to control amount of oxygen in boiler feed waters either by chemical or physical methods.

Principle : Oxygen present in sample oxidizes the divalent manganeous to its higher valency which precipitates as a brown hydrates oxide after addition of NaOH and KI. Upon acidification, manganese reverts to divalent state and liberates iodine from KI equivalent to DO content of the sample. The liberated iodine is titrated against standard (N/80) solution of sodium thiosulphate using starch as an indicator.

Interference : Ferrous ion, ferric ion, nitrite, microbial mass and high suspended solids constitute the main sources of interference. Modifications in the estimation procedure to reduce these interferences are described in the procedure.

Apparatus

1. BOD bottles capacity 300 ml.
2. Sampling device for collection of samples.

Reagents

1. **Manganese sulphate :** Dissolve 480 gm tetrahydrate manganeous sulphate and dilute to 1000 ml. Filter if necessary. This solution should not give colour with starch when added to an acidified solution of KI.

2. **Alkali iodide-azide reagent :** Dissolve 500 gm NaOH and 150 gm KI and dilute to 1000 ml. Add 10 gms NaN_3 dissolved in 40 ml distilled water. This solution should not give a colour with starch solution when diluted and acidified.

3. H_2SO_4 Conc.

4. **Starch indicator :** Prepare paste or solution of 0.5 g starch powder in distilled water. Pour this solution in 100 ml boiling water. Allow to boil for few minutes and let settle overnight. Use clear supernate. Preserve with 1.25 gm salicylic Acid/l or by adding few drops of to toluene.

5. **Stock sodium thiosulphate 0.1 N :** Dissolve 24.82 $Na_2S_2O_3.5H_2O$ in boiled cooled distilled water and dilute to 1000 ml. Preserve by adding 5 ml chloroform or 1 gm NaOH per litre.

6. **Standard sodium thiosulphate 0.025 N :** Dilute 250 ml stock $Na_2S_2O_3$ solution to 1000 ml with freshly boiled and cooled distilled water. Preserve by adding 5 ml chloroform per litre. (This solution will have to be standardized against standard of dichromate solution for each set of titrations.)

Procedure :

1. Collect sample in a BOD bottle using DO sampler.
2. Add 2 ml $MnSO_4$ followed by 2 ml of NaOH + KI + NaN_3. The tip of the pipette should be below the liquid level while adding these reagents. Stopper immediately.
3. Mix well by inverting the bottle 2-3 times and allow the precipitate to settle leaving 150 ml clear. supernatent.
4. At this stage add 2 ml conc. $H2SO_4$. Mix well till precipitate goes into solution.
5. Take 203 ml of above solution in a conical flask and titrate against $Na_2S_2O_3$ using starch as on indicator. When 2 ml $MnSO_4$ followed by 2 ml NaOH + KI + NaN_3 is added to the sample as in step (2), 4.0 ml of original sample is lost. This 203 ml taken for titration will correspond to 200 ml of original sample.

$$200 \times 300/(200-4) = 203, \text{ml}$$

Calculations :

1 ml of 0.025 N $Na_2S_2O_3$ = 0.2 mg of O_2

$$\text{D.O. in mg/l} = \frac{(0.2 \times 1000) \times \text{ml of Thiosulfate}}{200}$$

Modification of DO Estimation Procedure

1. **Alsterberg azide modification :**

The method outlined earlier is known as Winkler Modification and also as Alsterberg Azide Modification. The reagent NaOH + KI + NaN_3 is used in the method to eliminate intergerence caused by NO_2. This also reduces interference due to higher concentration of ferric ions.

2. **Redeal Stewart Modification :**

This modification is used when the samples contain ferrous ion. Add 0.7 ml conc. H_2SO_4 followed by 1 ml 0.63% $KMnO_4$ immediately after

collection in the BOD bottle itself. If ferric ions are present in large concentration add 1 ml of 40% KF solution. Remove excess $KMnO_4$ using potassium oxalate just sufficient to neutralise $KMnO_4$ as excess oscalate give-negative error.

3. **Alum/flocculation Modification :**

Samples containing high suspended matter consume appreciable amount of 1° in acid condition. Therefore, the samples are treated as follows:

Add 10 ml of 10% alum solution followed by 1-2 ml of conc NH_4OH to 1000 ml of the sample. Allow to settle for 10 minutes and siphon the clear supernatent for DO estimation.

4. **Copper sulfate sulfamic acid flocculation modification :**

Activated sludge contain biological floc having high demand for O_2. Samples from such treatment plants are fixed by adding. 10.0 ml copper sulfate sulfamic acid reagent to 1000 ml sample. The reagent is prepared by adding 32 gm sulfamic acid to 475 ml distilled water + 50 gm $CuSO_4$ in 500 ml D. W.+25 ml acetic acid.

5. **Alkaline Hypochlorite Modification :**

To overcome interference of complex sulphur compound (waste water form sulphite pulp industry). Pretreatment with alkaline hypochlorite solution converts polythionates to sulphates and free sulphur.

Excess of hypochlorite is destroyed by addition of KI and sodium sulphite.

The test is difficult to perform and open to question.

SODIUM AND POTASSIUM

Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). The increased pollution of surface and ground water during the past decade has resulted in a substantial increase in the sodium content of drinking water in different regions of the world. Sewage, industrial effluents, sea water intrusion in coastal area, and the use of sodium compounds for corrosion control and water-softening processes all contribute to sodium concentration in water because of the high solubility of sodium salts and minerals. Sodium levels in ground water vary widely but normally range between 6 and 130 mg/l. In surface water the sodium concentration may be less than 1 mg/l or exceed 300 mg/l depending upon the geographical area.

The estimation of sodium and potassium is based on the emission spectroscopy, which deals with the excitation of electrons from ground state to higher energy state and coming back to its original state with the emission of light.

Principle : The sample solution is sucked by an atomiser under controlled conditions. The radiation from the flame enters a dispersing device in order to isolate the desired region of the spectrum. The intensity of isolated radiation can be measured by a phototube. After carefully calibrating the photometer with the solution of known composition and concentration, it is possible to correlate the intensity of a given spectral line of the unknown with the amount of an element present that emits the particular radiation.

Equipment :

Flame photometer. With flame accessories.

Reagents :

1. Deionised distilled water.
2. Stock sodium solution : Dissolve 2.542 g dry sodium chloride in 1000 ml distilled water.

   1 ml = 1.0 mg Na

3. Working sodium solution Dilute 10 ml of stock solution to 1 litre.

    1 ml = 0.1 mg Na.

4. Stock potassium solution : Dissolve 1.907 of dry (110°C)

    1 ml = 1 mg K

5. Working potassium solution : Dilute 10 ml of the stock solution to 1 litre.

    1 ml = 0.10 mg K

Procedure

1. For operating the instrument, follow the instructions given by the manufacturer.

2. Start the electrical supply and switch on the air compressor supply. Stabilize the air flow. The needle should be steady at the mark.

3. Switch on the gas and maintain the gas fuel mixture so that the blue flame is seen through the viewing window.

4. Aspirate the distilled water and adjust the galvanometer reading to zero.

5. Calibrate the instrument by aspirating the standard and adjusting the galvanometric reading to desire mark.

6. Aspirate distilled water to bring the reading to Zero mark.

7. Aspirate sample and note down the galvanometric reading .

8. Put off the fuel supply first followed by air and then main switch.

IRON

Being the fourth most abundant element by weight in the earth's crust, it occurs mainly in the divalent and trivalent state in water. The presence of iron in natural water can be attributed to the dissolution of rocks and minerals, acid mines drainage, landfill leachates, sewage and engineering industries.

The presence of iron in drinking water supplies is objectionable for a number of reasons. Under pH condition existing in drinking water supply, ferrous sulphate is unstable and precipitate as insoluble ferric hydroxide which settles out as a rust coloured silt. Such water often tastes unpalatable even at low concentration (0.3 mg/l) and stains loundry and plumbing fixtures. Iron also promotes the growth of 'Iron bacteria'. These microorganisms derive their energy from the oxidation of ferrous to ferric and in the process deposit a slimy coating on the piping.

Principle : The ferric form of iron is reduced to ferrous form by boiling with hydrochloric acid Hydroxylamine hydrochloride. Upon adding 1,10 phenanthroline (between pH 3.2 and 3.3) form a soluble chelated complex of orange red colour. Intensity of the colour is directly proportional to concentration of iron present in the sample.

Interference : Strong oxidizing agents such as CN, KO_2, polyphosphates, Cr, Zn in conc. exceeding 10 times the Fe conc. Co and Cu if 5 mg/l Ni if 2 mg/l colour and organic matter constitutes sources of interferences in the development of colour. Boiling with HCl and addition of hydroxylamine-hydrochloride remove interferences due to CN, PO_4 and other oxidizing agents. The metal ions get complexed with phenanthroline.

Apparatus

1. HCl conc.
2. Hydroxylamine HCl solution-dissolve 10 gm NH_2OH. HCl in 100 ml distilled water.

3. **Ammonium acetate buffer :** Dissolve 250 gm ammonium acetate in 150 ml distilled water. Add 700 ml conc. glacial acetic acid. Final volume will be slightly more than 1000 ml.

4. **Phenanthroline solution :** Dissolve 100 mg 1,10- phenanthroline monohydrate in 100 ml distilled water warm slightly or add 2 drops conc. HCl if necessary. 1 ml of this solution can chelate 100 mg iron.

5. **Stock iron solution :** Add 20 ml conc. H_2SO_4 to 50 ml distilled water and dissolve 1.404 gm Fe (NH_4) (SO_4) $2.6H_2O$ add dropwise 0.1 N $KMnO_4$ till faint pink colour persists. Dilute to 1000 ml. 1 ml = 200 µ g Fe.

6. **Standard iron solution :** Dilute 50 ml stock Fe solution to 1000 ml. Prepare this solution freshly. 1 ml = 10 µ g Fe.

Procedure

1. Take suitable aliquot about 50 ml (having 2 mg/l Fe) of well mixed sample in 125 ml conical flask.
2. Add 2 ml conc HCl followed by 1 ml Hydroxylamine-hydrochloride solution.
3. Add 2-3 glass beads and boil for 20-25 min. to ensure dissolution of Fe.
4. Cool to room temp. and transfer to nessler's tube.
5. Add 10 ml amm. acetate buffer and 2 ml 1,10 phenanthroline solution.
6. Dilute to 100 ml and mix well.
7. Prepare blank by substituting the sample by distilled water.
8. For soluble iron determination, take known vol. of filtered sample, acidify by adding 2 ml conc. HCl per 100 ml of sample and treat from step 5 onwards for colour development.
9. Prepare calibration curve taking standard iron solution in the same way in the range, 1,000-4,000 g/l with 1 cm light path.
10. Measure the developed colour after 10 min. at 510 nm.
11. Calculate the conc. of total or soluble Fe present in the sample from calibration curve and express as mg/l.

MANGANESE

The presence of manganese in natural water occurs in both dissolved and suspended forms. Fresh water may contain from one to several thousand micrograms of manganese per litre depending on the location. Higher levels of manganese are sometimes found in free flowing river water and usually associated with industrial pollution.

The presence of manganese in drinking water supplies may be objectionable for a number of reasons unrelated to health. Water with concentration exceeding 0.15 mg/l imparts undesirable taste. When manganese compounds in solution undergo oxidation, it is precipitated resulting in problems of incrustation. The growth of certain nuisance organisms is also supported by manganese. The organisms concentrate manganese and give rise to taste, odour and turbidity problems is the distributed water.

Principle : Manganese is oxidised to higher valence state (permanganic state) giving permanganitic colour by boiling with potassium periodate in acid condition and in the presence of silver nitrate catalyst. The colour is proportional to the manganese concentration.

Periodate method is more sensitive than persulphate in lower concentrations and colour produced is stable for longer time.

Interference : Chlorides, organic matter, iron, colour and turbidity interfere in the estimation of Mn.

Apparatus

1. Colorimeter to operate at 525 nm.
2. Nessler's tube cap. 100 ml.
3. Water bath.

Reagents

1. H_2SO_4 conc.
2. HNO_3 conc.

3. H_3PO_4 85%.
4. KIO_4 A.R. grade
5. $AgNO_3$ Salt
6. H_2O_2 30%
7. **Standard Mn solution :** Prepare 0.1 N $KMnO_4$ solution by dissolving 3.2 gm $KMnO_4$ in distilled water and making up to litre. Age for several weeks, filter and standardise against sodium oxalate. Calculate the volume of this solution necessary to prepare one litre of solution of such strength that 1.0 ml = 50.0 μ g Mn as follows.

$$\text{ml. } KMnO_4 \text{ Solution} = \frac{4.55}{\text{Normality of } KMnO_4}$$

To this vol. add 2-3 ml conc. H_2SO_4 and decolorize by adding $NaHSO_3$ dropwise (10%). Boil to remove excess SO_2. Cool and dilute to 1000 ml.

Procedure

1. In case, pretreatment is required take suitable aliquot of sample and add 5 ml conc. H_2SO_4 followed by 5 ml conc. HNO_3 evaporate till SO_3 fumes disappear.
   This will remove interfering radicals such as organi: :atter, chlorides and other oxidizable substances. This results in dehydration of silica and production of turbidity, cool it and filter.
2. Take filtrate add 85 ml distilled water, 5 ml HNO_3 and 5 ml H_3 PO_4 85%. Mix well. Addition of H_3PO_4 decolorizes Fe^{+++} ion and prevent precipitation of iodates or periodates of Mn.
3. If pretreatment is not required add 5 ml conc. H_2SO_4 5 ml conc. HNO_3 and 5 ml H_3PO_4 85% . Mix well.
4. Boil till sample concentrates to about 90 ml and cool.
5. Add 0.3 g KIO_4. If Mn concentration is 10 μ g or less add 20 mg $AgNO_3$.
6. Heat till boiling for 1 hr. Cool immediately and dilute to 100 ml.
7. Measure colour intensity at 525 nm.
8. Prepare a calibration curve using standard solution containing 50 to 1500 μ g of Mn/1.
9. Calculate the conc. of Manganese in the sample from calibration curve and express in mg/1 as Mn.

ALUM DOSE DETERMINATION

Jar Test

Chemical coagulation, flocculation and sedimentation together reduce suspended and colloidal solids, phosphates, fluorides, organic matter and certain toxicants. Alum, ferrous and ferric salts, when used for clarification result in producing better effluent than plain sedimentation. The exact doses of these coagulants cannot be theoretically calculated and therefore laboratory tests have to be carried out using the jar test procedure. This enables the investigations of such inter-related factors like pH, colour, turbidity, mineral matter, temperature, time of flocculation and the degree of agitation, which all control the coagulation and flocculation.

Principle Metal salts hydrolyse in presence of the natural alkalinity to form metal hydroxides. The multivalent cation can reduce the zeta-potential while the metal hydroxides are good adsorbents and hence remove the suspended particles by enmeshing them.

Procedure

1. Using 100 ml. of sample on a magnetic stirrer, add coagulant in small increments at a pH 6.0, after each addition, provide a 1 min. rapid mix followed by a 3 min., slow mix. Continue addition until a visible floc is formed. Use this dose for further experiments.
2. Take 1000 ml. sample in each of six beakers as shown in Fig.4.
3. Adjust the pH to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with standard alkali or acid. Add the predetermined dose of coagulant simultaneously to all beakers.
4. Give rapid mix for 1 min. to each sample; follow this with 14 min. flocculation at slow speed.
5. Measure turbidity or pertinent effluent concentration of each settled sample.
6. Plot the percent removal characteristics versus pH and select the optimum pH.

7. At this pH repeat steps 2, 4 & 5 varying the coagulant dosage.
8. Plot the % removal Vs. the coagulant dosage and select the optimum dose.
9. If a polyelectrolyte is used repeat the procedure, adding polyelectrolyte towards the end of the rapid mix.

Precautions

1. Add coagulant dosages simultaneously to all the beakers while stirring.
2. Add the dosages at the point where intimate mixing is ensured.
3. It is advisable to siphon out the settled sample from the beakers so as not to disturb the settled floc.
4. When using polyelectrolytes add them first before inorganic coagulant or as advised by the manufacturer.
5. Use a commercially available multiple stirrer with speed control. Fig.4.
6. Range of optimum pH values should be maintained for optimum utilization of the coagulant.

CHLORINATION

Chlorine is widely used for disinfection of water, for deodourization since it is a powerful oxidising agent and is cheaply available. It can be used in molecular form or in a hypochlorite form.

Principle : Chlorine combines with water to form hypochlorous and hydrochloric acid. Hypochlorous acid dissociates to give the OCl^- ion. Quantities of OCl^- and $HOCl^-$ depend on pH of the solution. Hypochlorites also give the OCl^- ions, $HOCl^-$ rupture the cell membranes of microbes - the disease producing organisms. These also react with the impurities like ammonia, oxidisable inorganic matter like ferrous ion, nitrites, etc. to form chloramines and stabler ions of the later respectively.

For effective disinfection, (a) dose of chlorine, (b) optimum contact period and (c) residual chlorine are required to be found out.

Interferences : Oxidisable organic and inorganic matter

A. **Determination of Available Chlorine** (Bleaching powder/Hypochlorite solution).

Idometric method

Available chlorine in bleaching powder liberates iodine from KI in acidic conditions which is then titrated with sodium thiosulphate using starch as an indicator.

Apparatus - Iodine flasks, burettes, pipette, etc.

Reagents :

1. Bleaching powder
2. Acetic acid concentrated
3. Potassium iodide crystals
4. Standard sodium thiosulphate 0.1N - Dissolve 25.0 gm $Na_2S_2O_3.5H_2O$ and dilute to 1000 ml. in freshly boiled and cooled distilled water. Add about 5 ml. chloroform as a preservative.

5. Starch indicator : Prepare slurry by adding small quantity of water to 0.5 gm starch powder. Add it to 100 ml boiling water and continue boiling for a few min. cool.
6. Standard chlorine solution : Weigh accurately 5.0 gm fresh bleaching powder and transfer to mortar. Add small quantity of water to prepare fine paste. Add some more water, stir and allow to settle for few min. Decant the supernatant. Repeat it till fine suspension is obtained. Dilute to 500 ml. Alternatively, dilute household hypochlorite solution which is more stable than a chlorine solution which can be obtained either from a chlorinator solution hose or by bubbling chlorine gas through distilled water. Standardise the solution before use.

Procedure

1. Pipette out 25 ml. suspension from 1 in conical flask.
2. Add a pinch of KI and sufficient distilled water (100 ml.).
3. Add 10 ml. acetic acid and allow the reaction to complete.
4. Titrate free iodine liberated with 0.1 N thiosulphate. Note the vol. required.
5. Prepare a reagent blank using distilled water. Note the volume of 0.1 N thiosulfate required for blank.
6. Calculate as follows:

1) Available chlorine $\text{mg/l} = \frac{(A-B)\ N \times 35450}{\text{ml sample}}$

where A = ml thiosulfate required by sample
B = ml thiosulfate required by blank
N = normality of thiosulphate
(Calculate % available chlorine from mg/l)

B. Determination of Chlorine Demand

Chlorine demand is the quantity of chlorine that is reduced or converted to inert or less active forms of chlorine by substances in the water.

In most cases, chlorine demand implies complete reaction with all chlorine-reactable materials and is defined as the difference between the amount of chlorine applied and the amount of free chlorine (hypochlorous acid, HOCl, or hypochlorite ion, OCl^-), remaining at the end of the contact period. The term "breakpoint chlorination" frequently is applied where only free chlorine remains after the contact period.

Chloramines can be destroyed by breakpoint or excess chlorination until a free chlorine residual, equal to the total chlorine, is attained, under some test conditions, particularly at low pH values, an apparent breakpoint chlorination is obtained in which free and combined chlorine coexist.

The chlorine demand varies with the amount of chlorine applied, time of contact, pH, and temperature. For comparative purposes, state all test conditions, including the method of determining residual chlorine. The smallest amount of residual chlorine considered significant is 0.1 mg. Cl. as Cl_2/l.

Apparatus

1. Required for estimation of residual chlorine.
2. Iodine flasks or stoppered bottles.

Note : Expose glassware to water containing atleast 10 mg. residual chlorine/l for 3 hrs. or more and rinse with distilled water before use.

Procedure

Reagents : as required in (A) & (C)

1. Take 100 to 250 ml. sample in 12 Iodine flasks or stoppered bottles.
2. Add standardised chlorine solution in ascending order. If chlorine demand of treated water is being estimated, doses from 0.0 to 300μ g Cl_2/l will be found useful. However, if the sample is polluted doses from 0.1 mg. to 3 mg. Cl_2/l may be required.

3. Allow a contact period of 30 minutes for potable water and suitably higher for polluted waters.

4. Estimate residual chlorine iodometrically as described under (A) determination of available chlorine or by other methods described under (C) determination of residual chlorine.

5. Plot residual chlorine versus chlorine, added. In case of organically polluted samples a distinct breakpoint can be obtained. But in case of treated water sample, it is possible that only a straight line is obtained in absence of any ammonium compounds (Fig.5).

6. A residual chlorine of 0.2 mg Cl_2/l after the breakpoint is recommended.

C. Determination of residual chlorine

The iodometric methods are suitable for measuring total chlorine concentration greater than 1 mg/l and hence generally used for standardization of chlorine solution to be applied for dosing. However, amperometric end point determination gives better sensitivity.

The DPD methods are operationally simpler for determining free chlorine than the amperometric titration. High concentrations of monochloramine interfere with the chlorine determination unless the reaction is stopped with arsenite or thioacetamide. In addition, the DPD methods are subject to interference by oxidized forms of manganese unless compensated for by a blank.

The free chlorine test, syringaldazine (FACTS, method) was developed as a procedure specific for free chlorine. It is unaffected by significant concentrations of monochloramines, dichloramine, nitrate, nitrite, oxidized forms of manganese and other interfering compounds. The method is most applicable and described below. Because of poor accuracy and precision and especially due to toxic nature of orthotoluidine, use of this method can not be encouraged, but being mostly adopted in water works as field test, the method is also discussed.

1. **Iodometric method** - As discussed in
   (A) - Determination of available chlorine
2. **DPD Ferrous titrimetric method**

Principle

N.N-diethyl-p-phenylene-diamine (DPD) is used as an indicator in the titrimetric procedure with ferrous ammonium sulphate (FAS). Where complete differentiation of chlorine species is not required, the procedure may be simplified to give only free and combined chlorine or total chlorine.

In the absence of iodide ion, free chlorine reacts instantly with DPD indicator to produce a red colour. Subsequent addition of a small amount of iodide ion acts catalytically to cause monochloramine to produce colour. Addition of iodide ion in excess evokes a rapid response from dichloramine. In the presence of iodide ion, part of the nitrogen trichloride (NCl_3) is included with dichloramine and part with free chlorine. A supplementary procedure based on adding iodide ion before DPD permits estimating proportion of NCl_3 appearing with free chlorine.

Bromine, bromamine, and iodine react with DPD indicator and appear with free chlorine.

For accurate results careful pH control is essential. At the proper pH of 6.2 to 6.5 the red colour produced may be titrated to sharp colourless end points. Titrate as soon as the red colour is formed in each step. In all methods for differentiating free chlorine from chloramines, higher temperatures increase the tendency for chloramines to react and lead to increased apparent free-chlorine results. Higher temperatures also increase colour fading. Minimum detectable concentration : Approximately 18 μ g Cl. as Cl_2/L.

Interference :

The most significant interfering substance likely to be encountered in water is oxidized manganese. To correct for this, place 5 ml. buffer

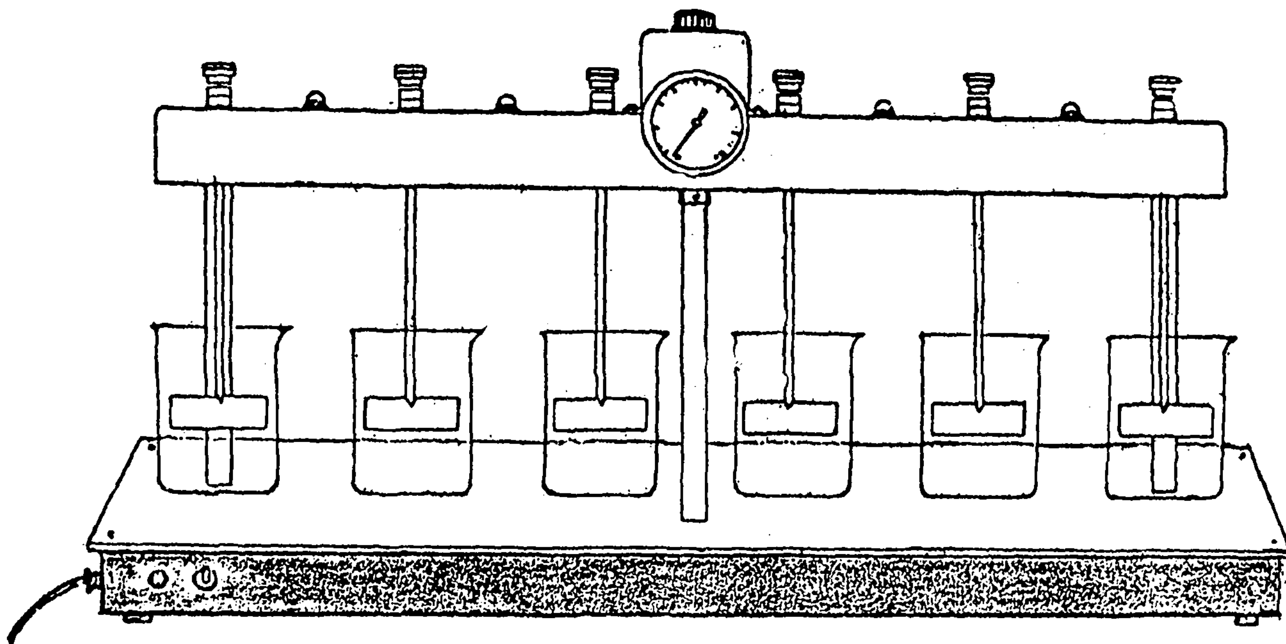


Fig.4: Multiple Stirrer With Floc Illuminator (Jar Testing Machine)

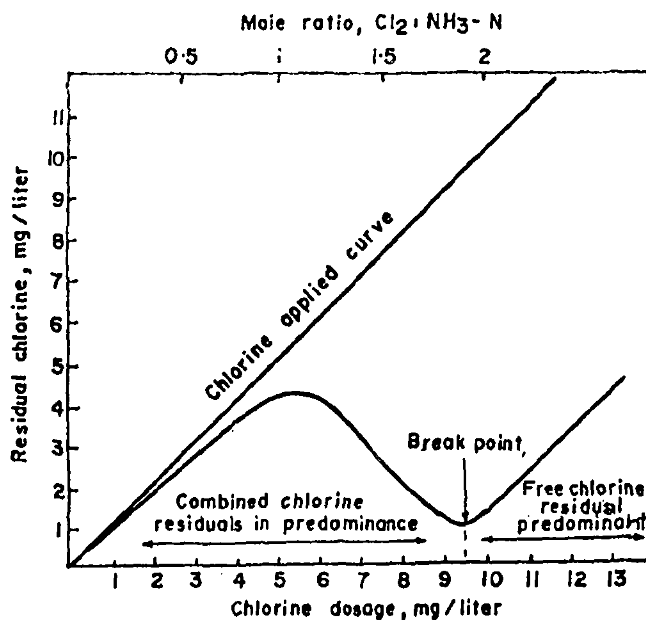


Fig.5: A Residual Chlorine Curve Showing A Typical Break Point (Ammonia-Nitrogen Content Of Water, 1.0 mg/l)

solution and 0.5 ml. sodium arsenite solution in the titration flask. Add 100 ml. sample and mix. Add 5 ml. DPD indicator solution, mix and titrate with standard FAS titrant until red colour is discharged. Subtract reading from Reading A obtained by the normal procedure of this method.

As an alternative to sodium arsenite use a 0.25% solution of thioacetamide, adding 0.5 ml. to 100 ml. sample.

Interference by copper of approximately 10 mg. Cu/1 is overcome by the EDTA incorporated in the reagents. EDTA enhances stability of DPD indicator solution by retarding deterioration due to oxidation, and in the test itself, provides suppression of dissolved oxygen errors by preventing trace metal catalysis.

High concentrations of combined chlorine can break through into the free chlorine fraction. At 10°C this amounts to 2% and at 25°C to 4% of the monochloramine present that reacts after standing 1 min. Adding thioacetamide (0.5 ml. 0.25% solution to 100 ml.) immediately after mixing DPD reagent with sample completely stops further reaction with combined chlorine in the free chlorine measurement. Continue immediately with FAS titration to obtain free chlorine. Obtain total chlorine from the normal procedure, i.e. without using thioacetamide.

Reagents

a. Phosphate buffer solution :

Dissolve 24 gm anhydrous Na_2HPO_4 and 46 anhydrous KH_2PO_4 in distilled water. Combine with 100 ml. distilled water in which 800 mg. disodium ethylenediamine tetraacetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water and add 20 mg. $HgCl_2$ to prevent mold growth and interference in the free chlorine test caused by any trace amounts of iodide in the reagents(CAUTION: $HgCl_2$ is toxic - take care to avoid ingestion).

b. N.N-Diethyl-p-phenylenediamine (DPD) indicator solution :

Dissolve 1 gm. DPD oxalate or 1.5 gm. DPD sulphate pentahydrate or 1.1 gm. anhydrous DPD sulphate in chlorine-free distilled water containing 8 ml. 1 + 3 H_2SO_4 and 200 mg. disodium EDTA. Make up to 1 1 L. store in a brown glass-stoppered bottle in the dark, and discard when discoloured. Periodically check solution blank for absorbance and discard when absorbance at 515 nm. exceeds 0.002/cm. (The buffer and indicator sulphate are available commercially as a combined reagent in stable powder form) CAUTION : The oxalate is toxic - take care to avoid ingestion.

c. Standard ferrous ammonium sulphate (FAS) titrant :

Dissolve 1.106 gm Fe $(NH_4)_2(SO_4)_2.6H_2O$ in distilled water containing 1 ml. 1 + 3 H_2SO_4 and make up with freshly boiled and cooled distilled water. This standard may be used for 1 month, and the titer checked by potassium dichromate (0.1 N). The FAS titrant is equivalent to 100 μ g Cl. as Cl_2/1.00 ml.

d. Potassium iodide, KI, crystals.

e. Potassium iodide solution :

Dissolve 500 mg. KI and dilute to 100 ml, using freshly boiled and cooled distilled water. Store in a brown glass-stoppered bottle preferably in a refrigerator. Discard when solution becomes yellow.

f. Sodium arsenite solution :

Dissolve 5.0 g $NaAsO_2$ in distilled water and dilute to 1 1 (CAUTION: Toxic - take care to avoid ingestion).

g) Thioacetamide solution :

Dissolve 250 mg. CH_3CSNH_2 in 100 ml. distilled water. (CAUTION : Cancer suspect agent. Take care to avoid skin contact or ingestion).

Procedure

The quantities given below are suitable for concentrations of total chlorine up to 5 mg/1. If total chlorine exceeds 5 mg/1, use a smaller

sample and dilute to a total volume of 100 ml. Mix usual volumes of buffer reagent and DPD indicator solution or usual amount of DPD powder, with distilled water before adding sufficient sample to bring total volume to 100 ml.(If sample is added before buffer, test does not work).

a. Free chlorine or chloramine :

Place 5 ml. each of buffer reagent and DPD indicator solution in titration flask and mix(or use about 500 mg. DPD powder). Add 100 ml sample, or diluted sample and mix.

1. **Free chlorine** - Titrate rapidly with standard FAS titrant until red colour is discharged (Reading A).

2. **Monochloramine** - Add one very small crystal of KI (about 0.5 mg.) or 0.1 ml (2 drops) KI solution and mix. Continue titrating until red colour is discharged again (Reading B).

3. **Dichloramine** - Add several crystals KI (about 1 mg) and mix to dissolve. Let stand for 2 min and continue titrating until red colour is discharged (Reading C). For dichloramine concentrations greater than 1 mg/l let stand 2 min more if colour drift back indicates incomplete reaction. When dichloramine concentrations are not expected to be high, use half the specified amount of KI.

4. **Simplified procedure for free and combined chlorine or total chlorine**- Omit 2) above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator, and titrate after 2 min standing.

b. Nitrogen trichloride :

Place one very small crystal of KI (about 0.5 mg.) or 0.1 ml KI solution in a titration flask. Add 100 ml sample and mix. Add contents to a second flask containing 5 ml each of buffer reagent and DPD indicator solution(or add about 500 mg DPD powder direct to the first flask). Titrate rapidly with standard FAS titrant until red colour is discharged (Reading N).

4. Calculation

For a 100 ml sample, 1.00 ml. standard FAS titrant = 100 mg. Cl as Cl_2

Reading	NCl, Absent	NCl_3 present
A	Free Cl	Free Cl
B-A	NH_2Cl	NH_2Cl
C-B	$NHCl_2$	$NHCl_2$ +
		1/2 NCl_3
N	-	Free Cl +
		1/2NCl_3
2 (N-A)	-	NCl_3
C-N	-	$NHCl_2$

In the event that monochloramine is present with NCl_3, it will be included in N, in which case obtain NCl_3 from 2(N-B).

Chlorine dioxide, if present, is included in A to the extent of one-fifth of its total chlorine content.

In the simplified procedure for free and combined chlorine, only A(free Cl) and C (total Cl) are required. Obtain combined chlorine from C-A.

The result obtained in the simplified total chlorine procedure corresponds to C.

3. Syringaldazine (FACTS) Method

Principle

The free (available) chlorine test, syringaldazine (FACTS) measures free chlorine over the range of 0.1 to 10 mg/l. A saturated solution of syringaldazine (3-5-dimethoxy-4 hydroxybenzaldazine) in 2-propanol is

used. Syringaldazine is stable when stored as a solid or as a solution in 2-propanol. It is oxidized by free chlorine on a 1.1 molar basis to produce a colored product with an absorption maximum at 530 nm. The color product is only slightly soluble in water; therefore, at chlorine concentrations greater than 1 mg/l, the final reaction mixture must contain 2-propanol to prevent product precipitation and color fading.

The optimum color and solubility (minimum fading) are obtained in a solution having a pH between 6.5 and 6.8. At a pH less than 6, color development is slow and reproducibility is poor. At a pH greater than 7, the color develops rapidly but fades quickly. A buffer is required to maintain the reaction mixture pH at approximately 6.7. Care should be taken with waters of high acidity or alkalinity to assure that the added buffer maintains the proper pH.

Temperature has a minimal effect on the color reaction. The maximum error observed at temperature extremes of 5 and 35°C is ≈ 10%. (Minimum detectable concentration : The FACTS procedure is sensitive to free chlorine concentrations of 0.1 mg/l).

Interferences

Interferences common to other methods for determining free chlorine do not affect the FACTS procedure. Monochloramine concentrations up to 18 mg/l dichloramine concentrations up to 10 mg/l, and manganese concentrations (oxidized forms) up to 1 mg/l do not interfere. Very high concentrations of nonchloramine (>35 mg/l) and oxidized manganese (2.6 mg/l) produce a color with syringaldazine slowly. Ferric iron can react with syringaldazine; however, concentrations up to 10 mg/l do not interfere. Nitrate (< 250 mg/l), nitrate (< 100 mg/l), sulphate (< 1000 mg/l), and chloride (1000 mg/l) do not interfere. Waters with high hardness (> 500 mg/l) will produce a cloudy solution that can be compensated for by using a blank. Oxygen does not interfere.

Other oxidizing agents, such as iodine, bromine, and ozone, will produce a color.

Apparatus

Spectrophotometer : for use at 530 nm. providing the light path of 1 cm for chlorine concentrations 1 mg/l or a light path from 1 to 10 mm for chlorine concentrations above 1 mg/l.

Reagents

a. **Chlorine-demand-free water** : Use to prepare reagent solutions and sample dilutions.

b. **Syringaldazine indicator** : Dissolve 115 mg. 3,5-dimethoxy-4-hydroxy-benzaldazine in 1 l 2-propanol.

c. **2-Propanol** : To aid in dissolution use ultrasonic agitation or gentle heating and stirring. Redistill reagent-grade 2-propanol to remove chlorine demand. Use a 30.5-cm (12-in.). Vigreux column and take the middle 75% fraction. Alternatively, chlorinate good-quality 2-propanol to maintain a free residual overnight; then expose to UV light or sunlight to dechlorinate.

d. **Buffer** : Dissolve 17.01 g KH_2PO_4 in 250 ml water; the pH should be 9.9. Mix equal volumes of these solutions to obtain FACTS buffer. pH 6.6. Verify pH with pH meter. For waters containing considerable hardness or high alkalinity other pH 6.6 buffers can be used, for example 23.21 g maleic acid and 16.5 ml 50% NaOH per litre of water.

Procedure

Calibration of photometer : Prepare a calibration curve by making dilutions of a standardized hypochlorite solution. Develop and measure colours as described below. Check calibration regularly , especially as reagent ages.

Free chlorine analysis : Add 3 ml sample and 0.1 ml buffer to a 5-ml capacity test tube. Add 1 ml syringaldazine indicator, cap tube, and

invert twice to mix. Transfer to a photometer tube or spectrophotometer cell and measure absorbance. Compare absorbance value obtained with calibration curve and report corresponding value as milligrams free chlorine per litre.

4. Orthotolidine Method

Principle : Orthotolidine is an aromatic compound. It gets oxidised by chlorine and produces a yellow coloured compound. This method measures a relative amount of free and combined available chlorine even in the presence of reducing inorganic substances with suitable modification as against iodometry. The method is more sensitive to lower concentration and gets affected by temperature and contact period. To obtain a proper colour, (a) the solution should be at a pH 1.3 or low during contact period; (b) the ratio of orthotolidine to chlorine must be at least 3:1; and (c) concentration of chlorine should not exceed 10 mg/l.

The orthotolidine-chlorine reaction is sensitive to residual chlorine concentrations as low as approximately 0.01 mg/l.

Interference : Interfering substances include nitrate, ferric compounds, manganic compounds, organic iron compounds, lignocellulose and algae. The effect of these substances is to increase the apparent residual chlorine content of the sample. Suspended matter interferes and should be removed by centrifuging prior to test. Compensation of turbidity and colour can also be accomplished by adding 1 or 2 drops of reducing agent containing an oxidizable sulphur group.

In chlorinated water containing no more than 0.3 mg/l iron, 0.01 mg/l, manganic manganese and 0.1 mg/l nitrite nitrogen, development of the characteristic yellow colour with Orthotoluidine may be accepted as being due to chlorine. It is recommended that orthotolidine - arsenite method (OTA) be exployed to determine the amount of any additional colour produced by the above interferences. When such additional colour is found, the quantity is deducted to correct chlorine values and the OTA test should be used as a routine procedure.

Apparatus

Colorimetric Equipment - 1) Spectrophotometer or filter photometer for use in the wavelength range of 400 to 490 nm and a light path of 1 cm or more.

2) Comparator, colour and turbidity compensating.

3) French square bottles, cap 1 or 2 Oz.

Reagents

1. **Orthotolidine Reagent** - Dissolve 1.35 gm. Orthotolidine dihydrochloride in 500 ml. distilled water. Add this solution with constant stirring to a mixture of 350 ml. distilled water and 150 ml. conc. HCl solution should be stored in amber bottles or in dark. Protect all times from direct sunlight. Use no longer than 6 months and maintain at normal temp.

2. **Sodium arsenite reagent** - Dissolve 5.0 gm. Sodium arsenite $NaAsO_2$ in distilled water and dilute to 1 lit. (caution-toxic, take care to avoid ingestion).

3. **Permanent colour standards,** commercially available discs along with comparator (chloroscope) or permanent standards prepared in the laboratory.

Procedure

i) **Addition of sample to reagent :** Use 0.5 ml orthotolidine reagent in 10 ml cells, 0.75 for other volumes. Place the orthotolidine reagent in the nessler tube, colorimeter cell or other container add the sample to the proper mark or volume and mix.

ii) **Colour Development and Comparison :** Compare the colours of the sample and standards at the time of maximum colour development. (If the potable sample contains predominantly free chlorine the maximum colour appears almost instantly and begins to fade. Samples containing combined chlorine develop their maximum colour at a rate that is largely dependent on temperature, although the nitrogenous compounds present may influence this rate. Usually at 20°C maximum

colour develops in about 3 min; at 25°C, in about 2.5 min; and at 0°C, in about 6 min. About 5 min. after maximum colour develops, a slight fading begins. (Therefore, samples containing combined chlorine should be read within 5 min. and should, preferably, be allowed to develop colour in the dark.) When colour comparison is made against chromate-dichromate standards, use the same cell depth for both samples and standards.

iii) **Compensation for Interference :** Compensate for the interference due to the presence of natural colour or turbidity in one of two ways : (1) View the sample and standard horizontally after placing an untreated sample of the same thickness of clean water behind the sample under comparison; or (2) add 1 or 2 drops (0.05 to 0.1 ml) of decolourising solution to the developed chlorine-orthotolidine colour and mix until the yellow colour disappears (within a minute). Read the values from the calibration curve as 'apparent chlorine' and interferences as chlorine and then substract. Alternatively, decolourise a portion of the developed sample and use to null the photometer, whereupon the chlorine value is obtained directly from the curve. In the presence of excessive turbidity, centrifuge the sample for a brief period to bring it within the nulling range of the photometer.

5. Orthotolidine - Arsenite (OTA) Method

Within the limitations specified, the OTA method permits measurement of the relative amounts of free available chlorine, combined available chlorine and colour due to interfering substances. The precision of the method depends upon strict adherence to (i) the recommended time intervals for the addition of reagents and (ii) the temperature of the sample. Since precision is affected by the relative concentration of free and combined available chlorine in the sample, the temp of the sample under examination should never exceed 20°C in free available chlorine determination. The precision of determination for this fraction improves with the decreasing temperature.

Apparatus - As mentioned in OT method.

Reagents - As mentioned in OT method.

Procedure : Sample and reagent volumes - Label three comparator cells or French square bottles "A", "B", and "C". Use 0.5 ml orthotolidine reagent in 10 ml cells, 0.75 ml in 15 ml cells and the same ratio for other volumes of sample. Use the same volume of arsenite solution as orthotolidine.

Free available chlorine : To cell A, containing orthotolidine reagent, add a measured volume of water sample. Mix quickly, and immediately (within 5 sec) add arsenic solution. Mix quickly again and compare with colour standards as rapidly as possible. Record the result (A) as free available chlorine and interfering colours.

Estimation of interference : To cell B, containing arsenite solution add a measured volume of water sample. Mix quickly, and immediately add orthotolidine reagent. Mix quickly again and compare with colour standards as rapidly as possible. Record the result (B_1). Compare with colour standards again in exactly 5 min. and record the result (B_2). The values obtained represent the interfering colours present in the immediate reading (B_1) and in the 5 min. reading (B_2).

Total available chlorine : To Cell C, containing orthotolidine reagent, add a measured volume of water sample. Mix quickly and compare with colour standards in exactly 5 min. Record the result (C) as the total amount of residual chlorine present and the total amount of interfering colours.

Measure photometrically the colours developed by following the four directions above and convert the readings to the proper chlorine values by referring to a calibration curve prepared by treating known chlorine concentrations in the same manner as the unknown samples.

Calculations

Total available residual chlorine = $C-B_2$

Free available residual chlorine = $A-B_1$

Combined available residual chlorine = Total available residual Cl-free available residual Cl.

ROUTINE BACTERIOLOGICAL ANALYSIS

General

Bacteriological analysis is carried out to assess the quality of raw as well as treated water, especially to detect faecal population. It enables to decide the adequacy of chlorination before the finished water is supplied to the consumers. In wastewater treatment, it may be carried out to determine the bacterial load contributed by treatment plant effluent to a receiving water body.

Bacteriological analysis includes estimation of (1) total bacterial count, (2) coliform count, (3) faecal coliforms, (4) E. coli, and (5) faecal streptococus. Differenciation of organisms may be done by gram staining.

Principle

The enumeration of microorganisms is usually carried out by MTD technique wherein biochemical reactions are used to detect the various groups mentioned above.

Apparatus

1. Autoclave
2. Oven for use at 160 to 180°C
3. Incubator for use at 44°C
4. Incubator for use at 37°C
5. Sampling device

Reagents

1. **Buffered Dilution Water** - To prepare a stock phosphate buffer solution, dissolve 34 grams of potassium dihydrogen phosphate (KH_2PO_4), in 500 ml. distilled water, adjust to pH 7.2 with 1 N NaOH and dilute to 1 litre with distilled water.

Add 1.25 ml. stock phosphate buffer solution to 1 litre distilled water. Dispense in amounts that will provide 18 ± 0.4 ml. or 9 ± 0.2 ml in 150 x 25 mm(6" x 1") or 150 x 18 mm (6" x 3/4") test tubes respectively. Sterilize in autoclave at 121°C for 15 min.

2. **Nutrient broth** - is a general purpose medium used for the growth of a variety of microorganisms.

Peptone	..	5 gms
Beef extract	..	3 gms
Distilled water	..	1000 ml

Adjust the pH 7.2 and distribute in required quantity. Sterilize at 121°C for 15 min, in the autoclave.

3. **Nutrient agar** - is a solid medium used for the growth and or enumeration of bacteria. To nutrient broth as in 2, add 1.5% agar. After adding the agar, steam for 15 minute, to allow to dissolve agar, steam for 15 minute, to allow to dissolve agar completely and sterilize at 121°C for 15 minutes in the autoclave.

4. **Macconkey broth** - This is used as a presumptive medium for the enumeration of coliform bacteria in water samples.

Peptone	..	20 gms
Lactose	..	10 gms
Sodium Chloride	..	5 gms
Bile salt	..	5 gms
Distilled water	..	1000 ml.

In place of bile salt, which is a commercial product, one can use sodium taurocholate or sodium tauroglycocholate.

Dissolve all the ingredients and adjust the pH to 7.4 After adjusting the pH, add 1 ml of 1% alcoholic solution of Bromocresol purple or 5 ml of 1 % aqueous solution of neutral red. This will be the single strength medium. Distribute 6 to 7 ml of the medium into 150 x 15 mm (6"x 5/8") test tubes and add a Durham's tube, 25 x 5mm

(1" x 3/16"). Plug the tubes with non-absorbent cotton and sterilize at 115°C for 10 minutes in the autoclave. This medium is used for 1 ml and the decimal dilutions of the water samples. For 10 ml and larger aliquots, a double strength medium is used. For the double strength medium add the above ingredients in double the quantities in 1000 ml of distilled water. This medium is dispensed into 10 ml quantities in 150 x 18 mm (6" x 3/4") test added with Durham's tube.

5. **Brilliant green bile lactose broth (BGB)** - This medium is used in a confirmatory test for coliforms as well as for califorms.

Peptone	..	10 gms
Lactose	..	10 gms
Bile salt	..	20 gms
Distilled water	..	1000 ml.

Dissolve all the ingredients and adjust the pH to 7.4 Add 1.33 ml of 1% aqeuous solution of brilliant green indicator. Distribute 4 ml quantities into 150 x 12 mm (6" x 1/2 ") test tubes and add a Durham's tube to each. After plugging with non-absorbent cotton, sterilize at 121°C for 15 minutes in the autoclave.

6. **Peptone water** - This is used for indole test or for preparing a liquid culture of an organism.

Peptone	..	10 gms
Sodium Chloride	..	5 gms
Distilled water	..	1000 ml.

Dissolve all the ingredients. Adjust the pH to 7.4 . Distribute 4 ml medium into 110 x 12 mm (4" x 1/4") and plug with non-absorbent cotton. Sterilize in the autoclave at 121°C for 15 minutes.

7. **Azide dextrose broth (ADB)** - This is a presumptive test medium used for enumerating faecal streptococci in the water sample.

Tryptone or polypeptone	..	15 gms
Beef extract	..	4.5 gms
Glucose	..	7.5 gms
Sodium chloride	..	7.5 gms
Sodium azide	..	0.2 gms
Distilled water	..	1000 ml.

Distill all the ingredients and adjust the pH to 7.3 Dispense 6 to 7 ml of medium into 151 x 15 mm (6" x 5/8") test tubes and plug with non-absorbent cotton. Sterilize in the autoclave at 121°C for 15 minutes. This is a single strength medium and used for 1 ml aliquots and decimal dilutions. When 10 ml sample or more has to be innoculated, use double strength medium. this is prepared by using double the quantities given above 1000 ml of water. 10 ml of this double medium is put into each 150 x 18 mm (6" x 3/4) test tubes.

8. **Ethyl violet Azide broth (EVA)**- confirmatory medium used for enumerating faecal steptococci.

Typtone or Biosate	..	20 gms
Glucose	..	5 gms
Sodium Chloride	..	5 gms
Dipotassium hydrogen phosphate	..	2.7 gms
Potassium dihydrogen phosphate	..	2.7 gms
Sodium azide	..	0.4 gms
Distilled water	..	1000 ml

Dissolve all the ingredients and adjust the pH to 7.1 Add 1 ml of 0.83% alcoholic solution of ethyl violet. Dispense 10 ml medium into 150 x 18 mm (6" x 3/4") test tubes and plug with non-absorbent cotton. Sterilize the autoclave at 121°C for 15 minutes.

9. **M.Endo broth** - Medium is used for enumerating coliforms by membrane filter techniques.

Tryptone or Polypeptone	....	10 gms
Thiopeptone or Thiotone	....	5 gms
Casitone or Trypticase	....	5 gms

Yeast extract	..	1.5	gms
Lactose	..	12.5	gms
Sodium Chloride	..	5	gms
Dipotassium hydrogen phosphate	..	4.375	gms
Potassium dihydrogen phosphate	..	1.375	gms
Sodium laural sulphate	..	0.05	gms
Sodium disoxycholate	..	0.10	gms
Sodium Sulphite	..	2.1	gms
Basic fuchsin	..	1.05	gms

Dissolve the above ingredients in 1000 ml of distilled water containing 20 ml of ethyl alcohol (95%). Heat the medium to the boiling point. Do not heat for a long time or do not submit to steam under pressure. the final pH should be between 7.1 to 7.3

10. **Kovac's Reagent** - Used for indole test.

Paradimethylaminobenzaldehyde	..	5	gms
Amyl alcohol or n-Butanol (pure)	..	75	ml
Concentrated hydrochloric acid	..	25	ml

Dissolve 5 grams of Paradimethylaminobenzaldehyde in amyl alcohol and then add 25 ml of HCl. The reagent must be Yellowish in colour. Store in amber coloured glass stoppered bottle.

11. **Methyl red soultion** - Used for methyl red test. Dissolve 0.1 gm of methyl red in 300 ml of 95% ethyl alcohol dilute to 500 ml with distilled water. Store in amber coloured glass stoppered bottles.

12. **Alpha-Napthol solution** - Used in V.P. test. Dissolve 5 grams of purified Aphal-napthol in 100 ml of absolute alcohol. The soultion should be prepared freshly.

13. **Potassium hydroxide solution** - used in V.P. test. Dissolve 40 gms of KOH in 100 ml distilled water.

14. **Gram staining reagents**

i) Crystal violet is used as a primary stain.

Solution A

Crystal violet (85% dye content)	.....	2	gms
Ethyl alcohol (95%)	.....	20	ml

Solution B

Ammonium Oxalate	.....	0.2	gm
Water	.....	20	ml

Mix solutions A and B ordinarily in equal parts. It is sometimes found, however, that this gives so concentrated stain that gram-negative organisms do not properly decolorize. To avoid this, solution A should be diluted as much as ten times, and 20 ml of this diluted solution should be mixed with solution B.

ii) Lugol's iodine - Dissolve 1 gm of iodine crystals and 2 gm of potassium iodine in 300 ml of distilled water.

iii) Safranin is used as a counter stain - Dissolve 2.5 gm of Safranin dye in 100 ml of 95% ethyl alcohol solution. Add 10 ml of the alcoholic soultion of safranin to 100 ml of distilled water.

iv) Ethyl alcohol 95%

DIFFERENT MEDIA REQUIRED FOR BACTERIOLOGICAL ANALYSIS :

A medium used for bacterial growth will vary accordingly to the nutritional requirements of the particular type of organisms for which it is prepared. It should have the following characteristics :

a) It should contain all nutrients in suitable amounts required by the organisms.
b) It should not contain any chemical that would affect the growth of the desired organism.

c) Its pH should be favourable for the growth of the organisms.
d) It should be sterile before use.

The autotrophs (chemolithotrophs) can grow on media containing only inorganic substances. They can use inorganic carbon and nitrogen for the synthesis of their protoplasm.

The heterotrophs (chemo-organotrophs) require organic sources of carbon and nitrogen. They derive energy from the oxidation of organic compounds. They have simple or complex nutritional requirements depending upon the organism.

For routine laboratory cultivation of heterotrophs media are not prepared by combining specific pure chemicals but certain complex materials of biological origin i.e. peptone meat or yeast extract.

Common ingredients of a culture medium - (for heterotrophs)

1. Water is absolutely necessary. Distilled water is used for preparation of media.

2. Peptones - They furnish organic nitrogen. They are also good buffers. They may also serve as carbon sources.

3. Fermentable carbohydrates- Serve as carbon and energy source. They are also useful for identifying and classifying organisms by studying their ability to ferment different carbohydrates.

4. Meat or yeast extracts - They supply vitamins and growth promoting substances.

5. Sodium chloride - It is added to maintain a proper osmotic pressure.

6. Minerals - These are needed in traces. They are generally present in requisite amounts in yeast or meat exrtacts or otherwise added to synthetic medium.

STERILIZATION - DIFFERENT METHODS

Sterilization is a process of freeing objects or materials from all living organisms.

Sterilization is usually accomplished by exposure to heat for sufficient time to kill all kinds of organisms. Since microorganisms vary in their resistance to heat, it is necessary that sterilization methods should be adequate to kill the most resistant organisms. Bacterial spores are very resistant to heat. Thus sterilization should kill the spores. For sterilization of most media and apparatus, heat is applied in some appropriate form depending on the nature of the object to be sterilized. Heat is generally applied in two forms :

1. Dry heat (flame or hot air)
2. Moist heat (steam) - flowing or under pressure.

Sterilization by hot air requires a much higher temperature and longer time than by moist heat.

A. STERILIZATION BY DRY HEAT

1. **Sterilization in flame** : Innoculating loops or needles and point of forceps etc, are sterilized by this method.

2. **Sterilization by hot air** : Oven is used for this purpose. A temperature of 160°C for one hour is necessary for complete destruction of bacterial spores. The oven is operated at this temperature for 1 to 1.5 hours depending on the load. Hot air oven is routinely used in sterilizing petridish, pipettes, flasks and test tubes. Petridishes are placed in metal box or wrapped in kraft paper in handly numbers (8-10). Pippetes are pluged at mouth end by cotton, wrapped in kraft paper or put in metal containers. Empty flasks and tubes are stopped with cotton plugs and wrapped with paper. Cotton and paper will char if the temperature rises above 180°C.

B. STERILIZATION BY MOIST HEAT :

1. **Sterilization by steam under pressure** (autoclaving) - Sterilization is accomplished at 121°C for 15 minutes in an autoclave. An autoclave is a closed chamber in which steam is compressed and regulated to the degree of temperature rerquired. The temperature of the steam at 15 lb pressure is 121°C (at sea level). Liquid media in glass containers are sterilized by this method.

2. **Intermittent sterilization :** Intermittent sterilization is used for substances that would decompose on exposure to high temperature. Free flowing steam at 100°C is used for sterilization. The principle underlying this method is that first heating period kills all vegetative cells present. After a lapse of 24 hours the spores if present will germinate into vegetative cells. The second heating will again destroy all vegetative cells. It sometimes happens that all spores do not germinate into vegetative form before the second heating period. Therefore, additional 24 hour period is allowed to elapse to make sure that all spores have germinated into vegetable cells. Arnold sterilizer is used for this work. The apparatus is operated for 20 to 30 minutes on three successive days. This method is used for sugar media or gelatin media.

C. STERILIZATION BY FILTRATION :

1. Filters of unglazed porcelain or diatomaceous earth are used. The pores of such filters should be small enough to prevent passage of bacteria. They are also available in different grades of porosity.

2. Sintered glass filters : Small volumes can be filtered with little loss in volume.

3. Seitz filters : There will be some loss in volume. In seitz filter asbestos is used for effective filtration.

4. Membrane filters : Filters of 0.45 micron pore size are good.

Standard Plate count

In solid medium, counting of organisms depends on the fact that living cells will proceed to multiply and in time produce sufficient progeny to form a colony visible to naked eye. Since bacteria occur in water as single cell, pairs, chains or even dense clumps, not every individual living cell will develop into a seperate colony. The results are expressed as number of counts per ml. i.e. Bacteria per ml.

a) Prepartion and dilution : Samples should be shaken well. Required portion shall be withdrawn with a sterile pipette, introduced into the petridish or dilution tube.

b) Plating : A 1 ml, 0.1 or 0.01 ml or other suitable dilution to be used for plating should be placed in the petridish first. Then 10 to 15 ml of melted agar medium at a temperature of 43 to 45°C tolerable to the skin, should be added to the petri-dish. The agar and the sample should be thoroughly mixed over the bottom of the petridish by tilting and rotating the dish in figure of 8. The plate should be allowed to solidify and placed immediately in the incubator in an inverted position.

c) Incubation : The plates should be incubated at 37°C for 24 hours at 20°C for 48 hours.

d) Counting : In preparing plates, such amount of water or dilution should be planted which will give form 30 to 300 colonies on a plate. There should be always more plates for dilution, at least two. The results as reported shall be the average of all plates falling within limits. It is not desirable to plant more than 30 from 1 ml. sample, it is obviously to be disregarded. In practice, we do use the counts even if the of colonies in a plate are less than, 30, when chlorinated water samples are plated, when the number of colonies are more the 300 in a plate, we report the count as TNC that in a plate report the count as TNC (too numerous to count). Counting shall be done with an approved counting aid, such as colony counter. The reported

number of bacteria per ml shall not include more than two significant figures. For example of 158 as 160 whereas a count of 35 is recorded as 35 and counts shall be designated as "Standard plate count" at 37°C or 20°C.

Coliform Count by MTD Method.

The coliform group includes all of the aerobic and facultative anaerobic gram negative, non-spore forming rod shaped bacteria which ferment lactose with gas formation within 48 hours at 37°C. The standard test for the estimation of numbers of the coliforms group may be carried out either by the Multiple Tube Dilution (MTD) or Membrane Filter (MF) methods.

It is convenient to express the results of the examination of replicate and dilutions in term of "Most Probable Number "(MPN). this term is actually an estimate based on certain probability formulae. The MPN value for a given sample is obtained by the use of MPN tables. Standard practice in water analysis is to plant five tubes for each dilution and a minimum three different dilutions are employed. The results are to be recorded in the proper form.

Details of procedure : The water sample is shaken thoroughly before making dilutions or before innoculation.

a) Presumptive test :

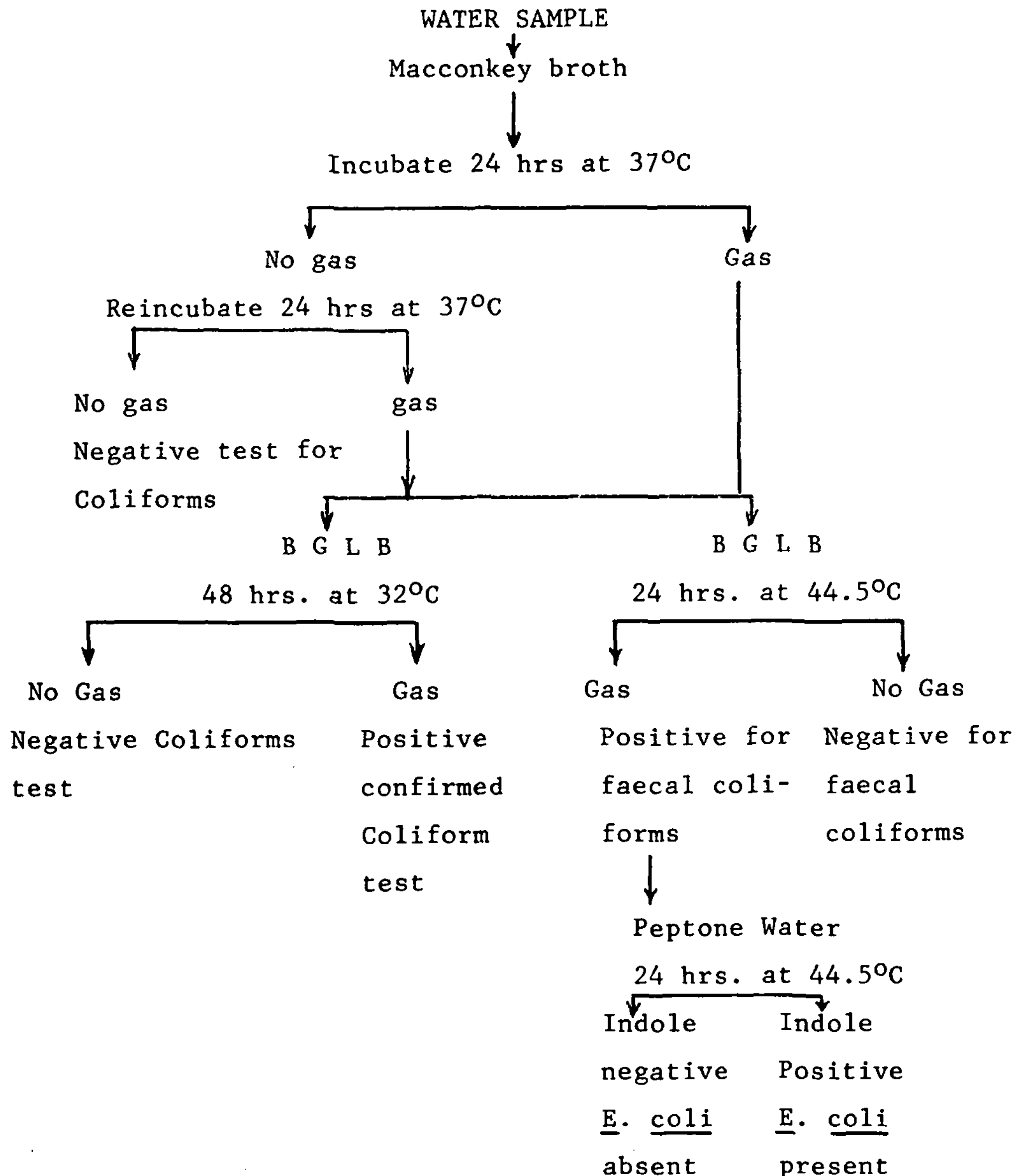
i) Macconkey broth may be used. Innoculate a series of fermentation tubes with appropriate measured quantities of nutritive ingredients in the mixture should be sufficient and according to the requirements. 10 ml and above aliquote should be innoculated in double strength and 1 ml and its should be inncoulated into single strength medium.

ii) Incubate all the tubes at 37°C for 24 hours to 48 hours. Examine each tube at the and of 24$\pm$2 hours. Examine for gas

Rapid test for E. coli

E. coli is one of the members of faecal coliforms, which ferments lactose with the produces indole from tryptophase at 44.5°C within 24 hours. Subculture from the positive tubes of BGLB or EC broth at 44.5°C (faecal coliforms) into tubes of peptone water. Incubate at 44.5°C for 24±2 hours. At the end of the incubation period test for indole production by adding a few drops of Kovacs' reagent. Positive test will give pink colour while negative test will give yellow colour.

ANALYSIS OF WATER FOR COLIFORMS FAECAL COLIFORMS AND E.COLI



Faecal streptococci count by MTD Method

Introduction : The terms faecal streptococci and enterococci have been used somewhat synonymously by many authors in recent years. The faecal streptococci group are indicators of faecal pollution of water because of general habitat of these organisms is the intestine of men and animal. They are gram positive cocci and ferment glucose with the production of acid only and are capable of growing in presence of 40% bile and at 45°C. On the basis of newer concepts of speciation of the faecal streptococci, it is suggested that the terms 'faecal streptococci' and Lancefields group D streptococcus' be considered as synonynous.

a) Presumptive test :

i) Innoculate a series of tubes of azide dextrose broth with appropriate graduated quantities of the water to be tested (follow the same precautions as given for coliforms).

ii) Incubate innoculated tubes at 37°C. Examine each tube at the end of 24 hours for the presence of turbidity. If no definite turbidity is present reincubate and read at the end of 48 hours.

b) Confirmed test

All azide dextrose broth tubes showing turbidity after 24 or 48 hour incubation must be subjected to the confirmed test. Transfer three loopful of growth from each azide dextrose broth to Ethyl Violet Azide broth tubes. Incubate the innoculated tubes for 48 hour at 37°C. The presence of streptococci is indicated by the formation of a purple button at the bottom of the tube, or occasionally by a dense turbidity. Find out the MPN value form the table. The results are recorded as numbers per 100 ml of the sample.

MF Technique for coliforms and streptococci

1. Description of MF assembly : There are many varieties of MF assemblies. The common one we have here is Millipore Standard Hydrosol

Filter Holder : Most components of this are made of stainless steel. These are locking ring, fine mesh stainless steel screen for supporting the filter membrane and the funnel assembly.

2. The membrane filters are obtained from millipore Filter Corporation (USA). They are HA type, white, grid marked, 47 mm with 0.45 micron pore size. There are membrane filters manufactured by other companies. These filters are used for many purposes.

3. Sterilization of filter assembly and filters is carried out at 121°C for 15 minutes and after sterilization, the steam in the autoclave should be released immediately by opening the outlet.

3. Test for coliform and streptococci

a) Selection of sample size : The size of the sample will be governed by the expected bacterial density. An ideal quantity will result in the growth of 20 colonies and not more than 200 colonies of all types. Samples should always be filtered in duplicate. If water is heavily contaminated use less quantity of water. When less than 20 ml is to be filtered, the portion should be diluted to a minimum volume of 30 ml before filtration.

b) Filtration of sample : Using sterile forceps, place a sterile filter, over the porous plant or stainless steel mesh of the apparatus, grid side up. Place the funnel unit carefully over the receptable and lock it in place. The filtration is then accomplished by passing the sample through the filter under vacuum. The filter should be rinsed by filtration, two to three times with 20 to 30 ml of sterile buffer water.

c) Unlock the assembly and remove the funnel and remove the filter by sterile forceps and place it on the sterile pad or agar with a rolling motion to avoid the enterapment of air.

i) Coliforms : The pad in a small petri dish is saturated with M. Endo broth and all filter is placed on it. Excessive medium is removed by tilting. The plates are inverted and incubated at 37°C for 24 hours under humid chamber.

All organisms which produce a dark red colony with a metallic sheen within 24 hour incubation are considered members of coliforms group and are counted. The count is made by a low power stereo-microscope.

$$\text{Coliform density} = \frac{\text{Coliform colonies counted} \times 100}{\text{ml of sample filtered}}$$

ii) Streptococci : Here instead of the pad, a solid agar medium is used. Pour approximately 10 ml of M-Enterococcus agar into 60 mm petri dishes. Allow to harden and place the filter on it. Invert and incubate at 37°C for 48 hour under humid chamber.

Count all red and pink colonies with the help of stereo-microscope. Express the count as number of faecal streptococci per 100 ml of water.

5. Advantages

a) Results are obtained within 24 hours as compared to MTD (48 to 96 hour).
b) Much larger voulme and hence more representative samples can be tested.
c) Get results with much greater precision.
d) Require less lab space, equipment and not bulky.
e) Involves less labour.

6. Limitations

a) Samples with more turbidity and less coliforms will be difficult to examine.
b) Samples having high numbers of non-coliforms gives less count.

DATA FOR RAPID CALCULATION OF THE LANGELIER INDEX

(Calcium Carbonate Saturation Index)

A		B		D	
Total Solids (ppm)	A	Galcium Hardness (ppm of $CaCO_3$)	C	M.O. Alkalinity (ppm of $CaCO_3$)	D
50-300	0.1	10-11	0.6	10-11	1.0
400-1000	0.2	12-13	0.7	12-13	1.1
		14-17	0.8	14-17	1.2
B		18-22	0.9	18-22	1.3
Temperature	B	23-27	1.0	23-27	1.4
(°F)		28-34	1.1	28-35	1.5
32-34	2.6	35-43	1.2	36-44	1.6
36-42	2.5	44-55	1.3	45-55	1.7
44-48	2.4	56-69	1.4	56-69	1.8
50-56	2.3	70-87	1.5	70-88	1.9
58-62	2.2	88-110	1.6	89-110	2.0
64-70	2.1	111-138	1.7	111-139	2.1
72-80	2.0	139-174	1.8	140-176	2.2
82-88	1.9	175-220	1.9	177-220	2.3
90-98	1.8	230-270	2.0	230-270	2.4
100-110	1.7	280-340	2.1	280-350	2.5
112-122	1.6	350-430	2.2	360-440	2.6
124-132	1.5	440-550	2.3	450-550	2.7
134-146	1.4	560-690	2.4	560-690	2.8
148-160	1.3	700-870	2.5	700-880	2.9
162-178	1.2	880-1000	2.6	890-1000	3.0

1. Obtain values of A,B,C and D from above table.
2. pHs =(9.3 + A + B) - (C + D).
3. Saturation Index = pH - pHs.
   If index is O, water is in chemical balance.
   If index is a plus quantity, scale forming tendencies are indicated.
   If index is a minus quantity, corrosive tendencies are indicated.

CONVERSION FACTORS

(Milligrams per Litre-Milli equivalents per Litre)

Ion (Cation)	Me/l=mg/lX	mg/l=me/lX	Ion(Anion)	me/l=mg/lX	mg/l=me/lX
Al^{3+}	0.1112	0.994	BO_2^-	0.02336	42.81
Ba^{3+}	0.2775	3.604	Br^-	0.01251	79.91
Ba^{2+}	0.01456	68.67	Cl^-	0.02821	34.45
Ca^{2+}	0.04990	20.04	CO_3^{2-}	0.03333	30.00
Cr^{3+}	0.05770	17.33	CrO_4^{2-}	0.01724	58.00
			F^-	0.05264	19.00
Cu^{2+}	0.03148	31.77	HCO^{-3}	0.01639	61.00
Fe^{2+}	0.03581	27.92	HPO_4^{2-}	0.02084	47.99
Fe^{3+}	0.05372	18.62	$H_2PO_4^{1-}$	0.01031	96.99
H^+	0.9921	1.08	HS^-	0.03024	33.07
K^+	0.02557	39.10	HSO_3^-	0.01233	81.07
			HSO_4^-	0.01030	97.07
Li^+	0.1441	6.939	I^-	0.007880	126.9
Mg^{2+}	0.08226	12.16	NO_2^-	0.02174	46.01
Mn^{2+}	0.03640	27.17	NO_3^-	0.01613	62.00
Mn^{4+}	0.07281	13.73	OH^-	0.05880	17.01
Na^+	0.04350	22.99	PO_4^{3-}	0.03159	31.66
NH_4^+	0.05544	18.04	S^{2-}	0.06238	16.03
Pb^{2+}	0.09653	103.6	SiO_3^{2-}	0.02498	38.05
Sr^{2+}	0.02283	43.81	SO_3^{2-}	0.02498	40.03
Zn^{2+}	0.03060	32.69	SO_4^{2-}	0.02083	48.03

FACTORS FOR PROBABLE COMPOSITION OF THE RESIDUE

	(a)	(b)	(c)	
1.	Ca x 1.497 = CO_3	CO_3 x 0.668 = Ca	Ca x 2.497 = $CaCO_3$	= CO_3 x 1.668
	Mg x 2.469 = CO_3	CO_3 x 0.405 = Mg	Mg x 3.469 = $MgCO_3$	= CO_3 x 1.405
	Na x 1.305 = CO_3	CO_3 x 0.766 = Na	Na x 2.305 = Na_2CO_3	= CO_3 x 1.766
	K x 0.767 = CO_3	CO_3 x 1.303 = K	K x 1.767 = K_2CO_3	= CO_3 x 2.303
2.	Ca x 2.397 = SO_4	SO_4 x 0.417 = Ca	Ca x 3.397 = $CaSO_4$	= SO_4 x 1.417
	Mg x 3.952 = SO_4	SO_4 x 0.253 = Mg	Mg x 4.952 = $MgSO_4$	= SO_4 x 1.253
	Na x 2.090 = SO_4	SO_4 x 0.479 = Na	Na x 3.090 = Na_2SO_4	= SO_4 x 1.479
	K x 1.228 = SO_4	SO_4 x 0.814 = K	K x 2.228 = K_2SO_4	= SO_4 x 1.814
3.	Ca x 1.769 = Cl	Cl x 0.565 = Ca	Ca x 2.769 = $CaCl_2$	= Cl x 1.565
	Na x 1.542 = Cl	Cl x 0.548 = Na	Na x 2.542 = NaCl	= Cl x 1.648
	Mg x 2.917 = Cl	Cl x 0.348 = Mg	Mg x 3.917 = $MgCl_2$	= Cl x 1.343
	K x 0.907 = Cl	Cl x 1.103 = K	K x 1.907 = KCl	= Cl x 2.103
4.	Ca x 3.094 = NO_3	NO_3 x 0.323 = Ca	Ca x 4.094 = $Ca(NO_3)_2$	= NO_3 x 1.323
	Mg x 5.100 = NO_3	NO_3 x 0.196 = Mg	Mg x 6.100 = $Mg(NO_3)_2$	= NO_3 x 1.196
	Na x 2.697 = NO_3	NO_3 x 0.371 = Na	Na x 3.697 = $NaNO_3$	= NO_3 x 1.371
	K x 1.586 = NO_3	NO_3 x 0.631 = K	K x 2.586 = KNO_3	= NO_3 x 1.631

CONVERSION OF HEAD ON THE WEIR TO FLOW

Discharge over 90° 'V' notch

Head in cm	Q m^3/hr	Head in cm	Q m^3/hr	Head in cm	Q $m^3/hr.$
1.0	0.055	17.5	65.255	34.0	336.562
1.5	0.151	18.0	69.957	34.5	348.920
2.0	0.3075	18.5	74.856	35.0	361.544
2.5	0.5336	19.0	79.953	35.5	374.435
3.0	0.828	19.5	85.229	36.0	384.520
3.5	1.225	20.0	90.752	36.5	397.847
4.0	1.696	20.5	96.459	37.0	414.735
4.5	2.27	21.0	102.375	37.5	428.7169
5.0	2.954	21.5	108.501	38.0	442.974
5.5	3.7411	22.0	114.841	38.5	457.510
6.0	4.635	22.5	121.395	39.0	472.327
6.5	5.652	23.0	128.168	39.5	487.425
7.0	6.787	23.5	135.160	40.0	502.807
7.5	8.048	24.0	142.375	40.5	518.474
8.0	10.114	24.5	149.814	41.0	534.428
8.5	10.964	25.0	157.477	41.5	550.671
9.0	12.626	25.5	165.374	42.0	567.203
9.5	14.421	26.0	173.499	42.5	584.028
10.0	16.3799	26.5	181.857	43.0	601.146
10.5	18.842	27.0	190.450	43.5	618.560
11.0	20.7147	27.5	199.280	44.0	636.270
11.5	23.133	28.0	208.350	44.5	654.278
12.0	25.697	28.5	217.660	45.0	672.58
12.5	28.423	29.0	227.214	45.5	691.196
13.0	31.315	29.5	237.013	46.0	710.109
13.5	34.374	30.0	247.060	46.5	729.326
14.0	37.605	30.5	257.355	47.0	748.850
14.5	41.010	31.0	267.902	47.5	768.681
15.0	44.592	31.5	278.702	48.0	788.822
15.5	48.354	32.0	289.756	48.5	809.273
16.0	52.298	32.5	301.068	49.0	830.037
16.5	56.428	33.0	312.638	49.5	851.115
17.0	60.746	33.5	324.469	50.0	872.507

WHO GUIDELINES FOR DRINKING WATER CONSTITUENTS

TABLE 1 - Microbiological and Biological Quality

Organism	Unit	Guideline value	Remarks
1. Microbiological quality			
A. Piped water supplies			
A1 Treated water entering the distribution system			
Faecal coliforms	Number/100 ml	0	turbidity 1 NTU : for disinfection with chlorine pH preferably 8.0 : free chlorine residual 0.2-0.5 mg/litre following 30 minutes (min) contact.
A2 Untreated water entering the distribution system			
Faecal coliforms	Number/100 ml	0	
Coliform organisms	Number/100 ml	0	In 98% of samples examined throughout the year-in the case of large supplies when sufficient samples are examined.
Coliform organisms	Number/100 ml	3	in an occasional sample, but not in consecutive samples.
A3 Water in the distribution system			
Faecal coliform	Number/100 ml	0	
Coliform organisms	Number/100 ml	0	In 95% of samples examined throughout the year-in the case of large supplies when sufficient samples are examined.

Contd./

Organism	Unit	Guideline value	Remarks
Coliform organisms	Number/100 ml	3	in an occassional sample but bot in consecutive samples.
B. Unpiped water supplies			
faecal coliforms	Number/100 ml	0	
coliform organisms	Number/100 ml	10	should not occur repeatedly if occurrence is frequent and if sanitary protection can not be improved, an alternative source must be found if possible.
C. Bottled drinking water			
faecal coliforms	Number/100 ml	0	source should be free from faecal contamination.
coliform organisms	Number/100 ml	0	
D. Emergency water supplies			
faecal coliforms	Number/100 ml	0	advise public to boil water in case of failure to meet guidelines values.
coliforms organisms	Number/100 ml	0	
Enter viruses	Number/100 ml	No guideline value set.	
II Biological quality		No guideline value set.	
protozoa (pathogenic)		- do -	
helminths (pathogenic)		- do -	
free-living organisms (algae others)		- do -	

TABLE - 2 : Inorganic Constituents of Health Significance

Constituents	Unit	Guideline value	Remarks
arsenic	mg/l	0.05	
asbestos	--	no guideline value set.	
barium	--	no guideline value set	
beryllium	--	- do -	
cadmium	mg/l	0.005	
chromium	mg/l	0.05	
cyanide	mg/l	0.1	
fluoride	mg/l	1.5	natural or deliberately added; local or climatic conditions may necessitate adaptation.
hardness	--	no health related guideline value set	
lead	mg/l	0.05	
mercury	mg/l	0.001	
nickel	--	no guideline value set	
nitrate	mg/l(N)	10	
nitrite	--	no guideline value set	
selenium	mg/l	0.01	
silver	--	no guideline value set	
sodium	--	- do -	

TABLE - 3 Organic Constituents of Health Significance

Constituents	Unit	Guide line Value	Remarks
aldrin & dieldrin	ug/1	0.03	
benzene	ug/1	10	
benzo pyrene	ug/1	0.01	
carbon tetra chloride	ug/1	3	tenative guide-line value
chlordane	ug/1	0.3	
chlorobenzene	ug/1	no health related guideline value set	odour threshold concentration between 0.1 and 3 ug/1
chloroform	ug/1	30	disinfection efficiency must not be compromised when controlling chlo-x roform content.
chlorophenols	ug/1	no health related guideline value set.	odour threshold concentration 0.1 ug/1
2,4-D	ug/1	100	
DDT	ug/1	1	
1,2 dichloroethane	ug/1	10	
1,1 dichloroethane heptachlor &	ug/1	0.3	
heptachlor epoxide	ug/1	0.1	
hexachlorobenzene	ug/1	0.01	
gamma-HCH(Lindane)	ug/1	3	
methoxychlor	ug/1	30	
Pentachlorophenol	ug/1	10	
Tetrachloroethene	ug/1	10	
Trichloroethene	ug/1	30	Tentative guide-line value
2,4,6 trichloro-phenol	ug/1	10	Odour threshold concentration 0.1 ug/1.
Trihalmethanes		no guide line value set.	See chloroform

TABLE - 4 Aesthetic Quality

Constituents of characteristic	Unit	Guideline value	Remarks
Aluminium	mg/l	0.2	
Chloride	mg/l	250	
Chlorophenols & Chlorobenzenes	--	no guideline value set	these compounds may affect taste and odour.
Colour	true colour (TCU)	15	
Copper	mg/l	1	
Detergents	--	no guideline value set	There should not be any foaming or taste & odour problems.
hardness	mg/l (as $CaCO_3$)	500	
hydrogen sulfide	--	not detectable by consumers	
iron	mg/l	0.3	
manganese	mg/l	0.1	
Oxygen dissolved	--	no guideline value set	
pH	--	6.5 - 8.5	
sodium	mg/l	200	
solids total dissolved	mg/l	1000	
sulfate	mg/l	400	
taste and odour	--	inoffensive to most consumers.	
temperature	--	no guideline value set	
turbidity	nephelometric turbidity units(NTU)	5	Preferably 1 for disinfection efficiency.
zinc	mg/l	5.0	

TABLE - 5 Radioactive constituents

Constituent	Unit	Guideline value	Remarks
Gross alpha activity	Bq/l	0.1	(a) If the levels are exceeded more detailed radionuclide analysis may be necessary.
gross beta activity	Bq/l	1	(b) Higher levels do not necessarily imply that the water is unsuitable for human consumption.

TABLE - 6 : Guidelines values for bacteriological quality

Refer table : 1 from A to D on page (v) and (vi)

INDIAN STANDARDS SPECIFICATIONS FOR DRINKING WATER
(IS:10500 - 1983)

Sl. No.	SUBSTANCE OR CHARACTERISTIC	METHOD OF TEST, CL REF OF IS: 3025-1964*	OTHER METHODS OF TEST	REQUIREMENT (DESIRABLE LIMIT)	UNDESIRABLE EFFECTS OUTSIDE THE DESIRABLE LIMIT	DESIRABLE/ ESSENTIAL	REMARKS
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i)	Colour, Hazen Units, Max	5	---	10	Above 10, consumer acceptance decreases	Essential	May be extended to 50 only if toxic substances are not suspected, in absence of alternate sources.
ii)	Odour	7	---	Unobjectionable	---	Essential	a) Test cold and when heated b) Test at several dilutions
iii)	Taste	Test at temperature not lower than 20°C. Test at several dilutions	---	Agreeable	---	Essential	Test to be conducted only after safety has been established
iv)	Turbidity, NTU, Max	---	Appendix B	10	Above 10, consumer acceptance decreases	Essential	May be extended up to 25, in absence of alternate sources
v)	Dissolved solids mg/l, Max	12	---	500	Beyond this palatability decreases may cause gastro intestinal irritation	Desirable	May be extended up to 3000, in the absence of alternate sources

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
vi)	pH value	8	---	6.5 to 8.5	Beyond this range the water will affect the mucous membrane and/ or water supply system	Essential	May be relaxed up to 9.2, in absence of alternate sources
vii)	Total hardness (as $CaCO_3$) mg/1, Max	16	---	300	Encrustation in water supply structure and adverse effects on domestic use	Essential	May be extended up to 600, in the absence of other sources
viii)	Calcium (as Ca) mg/1, Max	33	---	75	Encrustation in water supply structure and adverse effects on domestic use	Desirable	May be extended up to 200, in the absence of other sources
ix)	Magnesium (as Mg) mg/1, Max	16,33,34	---	30	Encrustation in water supply structure and adverse effects on domestic use	Desirable	May be extended up to 100, in the absence of other sources
x)	Copper (as Cu) mg/1, Max	36 (see Note)	---	0.05	Astringent taste,dis-coloration and corro-sion pipes, fittings and utensils will be caused beyond this	Desirable	May be relaxed up to 1.5
xi)	Iron (as Fe) mg/1, Max	32	---	0.3	Beyond this limit taste/appearance are affected, has adverse effect on domestic uses and water supply struc-tures & promotes iron bacteria	Essential	May be extended up to 1.0, in absence of alternate sources

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
xii)	Manganese (as Mn) mg/l, Max	35 (see Note)	---	0.1	Beyond this limit taste/appearance are effected, has adverse effect on domestic uses and water supply structures	Desirable	May be extended up to 0.5 where alternate source is not available
xiii)	Chlorides (as Cl) mg/l, Max	24	---	250	Beyond this limit, taste, corrosion & palatability are affected	Essential	May be extended up to 1000, in the absence of other alternate sources
xiv)	Sulphate (as SO_4) mg/l, Max	20	---	150	Beyond this causes gastro intestinal irritation when magnesium or sodium are present	Desirable	May be extended up to 400 provided magnesium (as Mg) does not exceed 30.
xv)	Nitrate (as NO_3) mg/l, Max	---	6 of IS:2488 (Part IV)-1974+	45	Beyond this methnaemoglobinemia, takes place	Desirable	No relaxation
xvi)	Fluoride (as F) mg/l,	23	---	0.6 to 1.2	Low fluoride levels are linked with dental caries. Above 1.5 it may cause fluorosis	Desirable	If the limit is below 0.6 water source should not be rejected but suitable public health measures should be taken. Maximum limit may be extended to 1.5, if no better alternate source is available

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
xvii)	Phenolic compounds (as C_6H_5OH), mg/l, Max	54	---	0.001	Beyond this, it may cause objectionable taste and odour	Desirable	May be relaxed up to 0.002
xviii)	Mercury (as Hg), mg/l, Max	---	(see Note) Mercury ion analyser	0.001	Beyond this, the water becomes toxic	Desirable	No relaxation of this limit is allowed. To be tested when pollution is suspected
xix)	Cadmium (as Cd), mg/l, Max	---	(see Note)	0.01	Beyond this, the water becomes toxic	Desirable	No relaxation of this limit is allowed. To be tested when pollution is suspected
xx)	Selenium (as Se), mg/l, Max	28 (see Note)	---	0.01	Beyond this, the water becomes toxic	Desirable	No relaxation of this limit is allowed. To be tested when pollution is suspected
xxi)	Arsenic (as As), mg/l, Max	40	---	0.05	Beyond this, the water becomes toxic	Desirable	No relaxation of this limit is allowed. To be tested when pollution is suspected
xxii)	Cyanide (*as CN*), mg/l, Max	---	Selective ion electrode method	0.05	Beyond this, limit the water becomes toxic	Desirable	No relaxation. To be tested when pollution is suspected
xxiii)	Lead (*as Pb*) mg/l, Max	---	(see Note)	0.1	Beyond this, limit, the water becomes toxic	Desirable	No relaxation being a health parameter. To be tested when pollution/plumbo-solvency is suspected

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
xxiv)	Zinc (as Zn) mg/l, Max	39	---	5	Beyond this, limit it can cause astringent taste and an opalescence in waters	Desirable	May be relaxed up to 15. To be tested when pollution is suspected
xxv)	Anionic detergents (as MBAS), mg/l, Max	---	Methyleneblue extraction method	0.2	Beyond this, limit it can cause a light froth in water	Desirable	May relaxed up to 1.0 To be tested when pollution is suspected
xxvi)	Chromium (as Cr^{6+}), mg/l, Max	38	---	0.05	May be carcinogenic above this limit	Desirable	No relaxation. To be tested when pollution is suspected
xxvii)	Polynuclear aromatic hydrocarbons (as PAH), mg/l, Max	++	++	---	May be carcinogenic	Desirable	++
xxviii)	Mineral oil mg/l, Max	---	Gas chromatographic method	0.01	Beyond this limit, undesirable taste & odour after chlorination takes place	Desirable	May be relaxed up to 0.03. To be tested when pollution is suspected
xxix)	Residual, free chlorine, mg/l, Min.	45	---	0.2	---	Essential	To be applicable only when water is chlorinated. Tested at consumer end. When protection against viral infectión is required, it should be Min 0.5 mg/l

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
xxx)	Pesticides	++	++	Absent	Toxic	Desirable ++	
xxxi)	Radioactive materials :	---	58		---	Desirable ---	
	a) Alpha emitters µ c/ml, Max			10^{-6}			
	b) Beta emitters µ c/ml, Max			10^{-7}			

NOTE - Atomic absorption spectrophotometric method may be use.

* Methods of sampling and test (physical and chemical) for water used in industry.

\+ Methods of sampling and test for industrial effluents, Part IV.

++ Limits and methods of test are under study.