NEW AGE

A COMPREHENSIVE LABORATORY MANUAL FOR ENVIRONMENTAL SCIENCE AND ENGINEERING

P. R. Sreemahadevan Pillai

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P. R. SREEMAHADEVAN PILLAI

Principal
P.A. Aziz College of Engineering & Technology
Thiruvananthapuram-695564
Kerala

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PREFACE

The awareness on environmental matters is spreading fast and attained importance on global scale, which has led to a better scope for a systematic study in Environmental Science and Engineering. In the last decade, a good number of undergraduate and postgraduate courses in environmental science and engineering has come up in most of the Universities in India, aiming at the tremendous employment potential in this area. In addition, the University Grants Commission has made it mandatory that at least one paper on environmental studies should be compulsorily included in the syllabus of any basic degree course in the country. Most of the newly begun undergraduate and postgraduate courses in Environmental Science and Engineering have laboratory sessions attached to it. Many students of these courses are missing the fundamentals necessary for conducting experiments in this field due to the non-availability of a standard reference book.

This book is basically meant for students, consultants, researchers and voluntary activists engaged in Environmental Science and Engineering field, who would like to learn all the fundamental experiments necessary for them. The book is designed to be a comprehensive reference manual as well as a practical record book. Since the different Universities have different syllabi for the same subject, all the common experiments are discussed here, so that students of any particular institution can resort to the selected experiments of their choice.

It is worth mentioning that digital meters are now available for most of the tests in this field. The difficulty with these instruments are that they require very costly consumable and reagents, which are mostly proprietary. In addition, their accuracy cannot be confirmed without frequent calibration. Hence the validity of these experiments will survive the test of time as cost-effective and popularly accepted ones. Moreover, any environmental scientist/ engineer is supposed to know the fundamental principles of the experiment, before resorting to sophisticated instruments.

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APPENDICES

GENERAL

I. Instructions

1. This laboratory manual is for reference and use for those using the environmental science/engineering laboratory.
2. Discussion after each experiment should be based on the following points:
   (i) Limit prescribed for that constituent in drinking water standards.
   (ii) The suitability of the sample for drinking purpose with respect to that particular constituent.
3. Users may refer the following for writing the discussion after each experiment:
   (i) *"Standard Methods for the Examination of Water and Waste Water"*, American Public Health Association, 1015, 15th Street, N.W., Washington D.C., 2005.
   (ii) *"Chemistry for Environmental Engineers"*, Sawyer and McCarty, Tata Mc-Graw Hill.
   (iii) *"Manual of Standards of Quality for Drinking Water Supplies",* Indian Council of Medical Research, New Delhi.
   (iv) *"International Standards for Drinking Water"* — World Health Organisation.
   (v) "IS 2490 - 1981, IS 3306 - 1974, IS 3307 - 1977, IS 7968 - 1976, IS 2296 - 1974*",* Bureau of Indian Standards, New Delhi.

II. DOs and DON'Ts in the Laboratory

1. Do thoroughly clean the glassware before and after use.
2. Do handle the glassware carefully.
3. Do not handle chemicals with bare hands.
4. Do not blow out the last drop from the pipette. When the liquid has drained out completely, touch the tip of the pipette to the inner surface of the vessel.
5. Do not add water to acids. Do always add acid to water.
6. Do use large volumes of water, when a person is splashed with acid to prevent serious burns.
7. Do weigh the articles in a balance only at room temperature.
8. Do use different pipette for different reagents.

9. Do not pipette out acids and other toxic reagents by mouth.
10. Do read the level of the curve (meniscus), in all volumetric glassware, with the eye at approximately the same level as the curve of solution.

III. General Information

In water and wastewater analysis, the results are usually reported in terms of mg/L of some particular ion, element or compound. It is most convenient to have the standard titrating agent of such strength, that 1mL is equivalent to 1mg of material being measured. Thus 1 litre of the standard solution is usually equivalent to 1g of the standard substance.

Normality

The desired normality of the titrant is obtained by the relationship of 1 to the equivalent weight of the measured material. Thus normality of acid solution to measure ammonia, ammonia nitrogen, and alkalinity as $CaCO_3$

Ammonia	—	1/eq. wt.	=	1/17	=	N/17	= 0.0588N
Ammonia N	—	1/eq. wt.	=	1/14	=	N/14	= 0.0715N
Alkalinity	—	1/eq. wt.	=	1/50	=	N/50	= 0.020N

The normality of basic solution to measure mineral acidity as $CaCO_3$ is:

Acidity	—	1/eq. wt.	=	1/50	=	N/50	= 0.020N

The normality of silver nitrate to measure chloride and sodium chloride is:

Chloride	—	1/eq. wt.	= 1/35.45		= N/35.45	=	0.0282N
Sodium chloride	—	1/eq. wt.	= 1/58.44		= N/58.44	=	0.071N

Thus the substance measured is calculated as follows:

$$= \frac{\text{mL of titrant used} \times 1,000}{\text{mL of sample}} \text{ mg/L}$$

Most materials subjected to the analysis of water and wastewater fall in the realm of dilute solutions i.e., a few mg in a litre. So the results are normally expressed in mg/L or ppm. Parts per million (ppm) is a weight ratio; but mg/L is a weight by volume ratio. The relationship is given as follows:

$$\text{ppm} = \frac{\text{mg/L}}{\text{Sp.gr.}}$$

If concentrations are less than 0.1 mg /L, express them in µg/L (micrograms per litre). If concentrations are more than 10,000 mg/L, they are expressed in percentages.

Plotting of Graphs

Rules listed by Worthing and Geffner are to be followed while plotting graphs. They are:

1. The independent and dependent variables should be plotted on abscissa and ordinate respectively.
2. The scale should be so chosen that the value of either coordinate could be found quickly and easily.
3. The curve should cover as much of the graph sheet as possible.
4. The scales should be so chosen that the slope of the curve approach unity as nearly as possible.
5. The variables should be chosen to give a plot that will be as nearly a straight line as possible.

Classification of Procedures

Laboratory analytical procedures are classified to quantify the chemical substances as follows:

1. Toxic chemical substances: e.g., lead, arsenic, selenium, hexavalent chromium, cyanide.
2. Chemical substances affecting health: e.g., fluoride, nitrate.
3. Chemical substances affecting potability: e.g., residue, turbidity, colour, taste and odour, iron, manganese, copper, zinc, calcium, magnesium, sulphate, chloride, pH and phenolic compounds.
4. Chemical substances indicative of pollution: e.g., total organic matter, BOD, Kjeldahl nitrogen (total organic nitrogen), albuminoid nitrogen, nitrite nitrogen and phosphate.
5. Residual chlorine.

Standards of Water Quality

Standards of water quality are presented as follows:

Bacteriological Quality

1. **Treated water:** In 90% of the samples examined throughout the year, the coliform bacteria shall not be detected or the MPN index shall be less than 10. None of the samples shall have an MPN index of coliform bacteria in excess of 10. An MPN index of 8–10 shall not occur in consecutive samples.
2. **Untreated water:** In 90% of the samples examined throughout the year, the MPN index of coliform organisms should not be less than 10. None of the samples should show an MPN index greater than 20. An MPN index of 15 or more should not be permitted in consecutive samples.

Chemical and Physical Quality

Classification	*Substances*	*Maximum allowable concentration*
1. Toxic substances	Lead (Pb)	0.1 mg/L
	Selenium (Se)	0.05 mg/L
	Arsenic (As)	0.2 mg/L
	Chromium (Cr^{6+})	0.05 mg/L
	Cyanide (CN)	0.01 mg/L
2. Chemical substances which may affect health	Fluoride (F^-)	0.08–1.0 mg/L
	Nitrate (NO_3^-)	50.0 mg/L
	Total solids	500 mg/L
	Colour	5 Units
	Turbidity	5 Units
	Taste	Unobjectionable
	Odour	Unobjectionable
	Manganese (Mn)	0.1 mg/L

Contd...

3. Chemical substances affecting the potability of water	Iron (Fe)	0.3 mg/L
	Copper (Cu)	1.0 mg/L
	Zinc (Zn)	5.0 mg/L
	Calcium (Ca)	75 mg/L
	Magnesium (Mg)	50 mg/L
	Sulphate	200 mg/L
	Chloride (Cl^-)	200 mg/L
	pH range	7.0–8.5
	Phenolic substances	0.001mg/L

Significance and Determination of Chemical Parameters

Chemical parameters and their significance are presented as follows. The methods of the analysis adopted are also presented. However, only simple methods will be dealt within this manual.

Chemical parameters commonly determined in natural waters and water supplies

No.	*Chemical species*	*Significance in water*	*Methods of analysis commonly used*
1.	Acidity	Indicative of industrial pollution, acid mine drainage	Titration
2.	Alkalinity	Water treatment, buffering, algal productivity	Titration
3.	Ammonia	Productivity, pollution	Colorimetry
4.	Calcium	Hardness, productivity treatment	Atomic absorption
5.	Carbon dioxide	Bacterial action, corrosion	Titration, calculation
6.	Chloride	Saline water contamination	Titration, potentiometry
7.	Chlorine	Water treatment	Colorimetry, titration
8.	Fluoride	Water treatment, toxic at high level	Colorimetry, potentiometry
9.	Hardness	Water quality, treatment	Titration, atomic absorption
10.	Iron	Water quality, treatment	Colorimetry, atomic absorption
11.	Magnesium	Hardness	Atomic absorption
12.	Manganese	Water quality	Atomic absorption
13.	Nitrate	Productivity, toxicity	Colorimetry, potentiometry
14.	Nitrite	Toxic, pollutant	Colorimetry

Contd...

15.	Nitrogen (albumin.)	Proteinaceous material	Colorimetry
16.	Nitrogen (organic)	Organic pollution	Colorimetry
17.	Oxygen	Water quality	Titration, electrochemical
18.	BOD	Water quality, pollution	Microbiological titration
19.	COD	Water quality, pollution	Chemical oxidation-reduction
20.	pH	Water quality, pollution	Potentiometry
21.	Phosphate	Water quality, pollution	Colorimetry
22.	Sulphate	Water quality, pollution	Gravimetry, turbidimetry
23.	Sulphide	Water quality, pollution	Colorimetry, potentiometric titration

IV. Introduction

Humanity and Environment

A characteristic, which has set *Homo sapiens* apart from other species, has been their ability to control many aspects of their environment. Throughout recorded history people have continually struggled to manage their natural environment in order to improve their health and well-being. In recent years environmental sanitation in many parts of the world has led to large reduction or virtual elimination of diseases spread via the environment. Continuous environmental vigilance is necessary to keep away these weeds from the garden of humanity from increasing out of proportion among a large part of the earth's population.

People's success in the control of environmental borne diseases has not reduced the need for ever-increased efforts of effective management of the total environment. The population explosion, an affluent society with desires for a vast array of products, increased radiations, greater energy use, increased food production needs, and other developments have created strains on parts of the ecological systems. Perhaps never in history have people demonstrated such great concern for their total environment as is now being witnessed in many parts of the earth, particularly in those areas which have benefited most from people's environmental control efforts toward effective use of human, material and natural resources. Over the years, intensification of old problems and the introduction of new ones have led to basic changes in the philosophy of environmental engineering practice.

Water is one of the materials required to sustain life and has long been suspected of being the source of many of the illnesses of man. It was not until a little over 100 years ago that definite proof of disease transmission through water was established. Originally the major objectives were to produce hygienically safe water supplies and to dispose off wastes in a manner that would prevent the development of nuisance conditions. Many other factors concerned with aesthetics, economics, recreation and other elements of better living are important considerations and have become part of the responsibilities of the modern environmental engineer.

The public has been more exacting in their demands as time has passed, and today water engineers are expected to produce finished waters that are free of colour, turbidity, taste, odour and harmful metal ions. In addition, the public desires water, which is low in hardness and total solids, non-corrosive, and non-scale forming. To meet with such stringent standards, chemists, biologists and engineers must combine their efforts and talents together and hence the need for analytical testing of water and waste becomes necessary.

Importance of Quantitative Analysis

Quantitative analysis serves as the keystone of engineering practice. Environmental engineering is perhaps most demanding in this respect, for it requires the use of not only the conventional measuring devices employed by engineers but, in addition many of the techniques and methods of measurement used by chemists, physicists and some of those used by biologists.

Every problem in environmental engineering must be approached initially in a manner that will define the problem. This approach necessitates the use of analytical methods and procedures in the field and laboratory, which have proved to yield reliable results. Once the problem has been defined quantitatively, the engineer is usually in a position to design facilities that will provide a satisfactory solution.

After construction of the facilities has been completed and they have been placed in operation, usually constant supervision employing quantitative procedure is required to maintain economical and satisfactory performance. The increase in population density and new developments in industrial technology are constantly intensifying old problems and creating new ones. In addition, engineers are forever seeking more economical methods of solving old problems. Research is continuously under way to find answers to the new problems and better answers to old ones. Quantitative analysis will continue to serve as the basis for such studies.

Character of Problems

Most problems in environmental engineering practice involve relationships between living organisms and their environment. Because of this, the analytical procedures needed to obtain quantitative information are in often a strange mixture of chemical and biochemical methods, and interpretation of the data is usually related to the effect on microorganisms or human beings. Also, many of the determination used fall into the realm of microanalysis because of the small amounts of contaminants present in the samples. Ordinarily, the amounts determined are a few milligrams per litre and often they are found only in few micrograms.

Standard Methods of Analysis

Concurrent with the evaluation of environmental engineering practice, analytical methods have been developed to obtain the factual information required for the resolution and solution of problems. In many cases different methods have been proposed for the same determination, and many of them were modified in some manner. As a result, analytical data obtained by analysis were often in disagreement.

In an attempt to bring order out of chaos, the American Public Health Association appointed a committee to study the various analytical methods available and published the recommendation of the committee as "Standard Methods of Water Analysis" in 1905.

"Standard Methods" as published today is the product of the untiring effort of hundreds of individuals who serve on committees, testing and improving analytical procedures for the purpose of selecting those best suited for inclusion in "Standard Methods", which is now available as "Standard methods for the examination of water and waste water".

Experiment No. _____ **Date** _____

DETERMINATION OF SOLIDS

Aim

The aim of the experiments is to determine the following types of solids in the given sample(s):

(a) Total solids
(b) Total (inorganic) fixed solids
(c) Total volatile (organic) solids
(d) Total dissolved solids
(e) Dissolved fixed (inorganic) solids
(f) Dissolved volatile (organic) solids
(g) Total suspended solids
(h) Suspended fixed (inorganic) solids
(i) Suspended volatile (organic) solids
(j) Settleable solids

Principle

'Total solids' is the term applied to the material left in the vessel after evaporation of a sample of water/waste water and its subsequent drying in an oven at a definite temperature. Total solids include "total suspended solids" the portion of total solids retained by a filter and "total dissolved solids" the portion that passes through the filter. Fixed solids is the residue remaining after ignition for 1 hour at 550°C. The solid portion that is volatilised during ignition is called volatile solids. It will be mostly organic matter. Waters that are low in organic matter and total mineral content and are intended for human consumption may be examined under 103–105°C or 179–181°C. But water containing considerable organic matter or those with pH over 9.0 should be dried at 179–181°C. In any case, the report should indicate the drying temperature.

The sample is filtered and the filtrate evaporate in a weighed dish on a steam bath, the residue left after evaporation is dried to constant weight in an oven at either 103–105°C or 179–181°C. The increase in weight over that of the empty dish represents total dissolved solids and includes all materials, liquid or solid, in solution or otherwise, which passes through the filter and not volatilised during the drying process.

The difference between the total solids and the total dissolved solids will give the total suspended solids. The dishes with the residue retained after completion of the tests for total solids and total dissolved solids are subjected to heat for 1 hour in a muffle furnace held at 550°C. The increase in weight over that of the ignited empty vessel represents *fixed solids* in each instance.

The difference between the total dissolved/total suspended solids and the corresponding fixed solids will give *volatile solids* in each instance. All the quantities should be expressed in mg/L. Settleable matter in surface and saline waters as well as domestic and industrial wastes may be determined and reported on a volume basis as millilitre per litre.

Apparatus

1. Porcelain evaporating dishes of 150–200 mL capacity
2. Steam bath
3. Drying oven
4. Desiccators
5. Analytical balance or monopan balance
6. Filter paper (preferably of glass fibre)
7. Electric muffle furnace
8. Imhoff cone

Procedure

(a) Total solids

1. Ignite the clean evaporating dishes in the muffle furnace for 30 minutes at 550°C and cool in a desiccator.
2. Note down the empty weight of the dish (W_1).
3. Pour a measured portion (50 to 100 mL) of the well-mixed sample into the dish and evaporate the contents by placing the dish on a steam bath.
4. Transfer the dish to an oven maintained at either 103–105°C or 179–181°C and dry it for 1 hour.
5. Allow the dish to cool briefly in air before placing it, while still warm in a desiccator to complete cooling in a dry atmosphere.
6. Weigh the dish as soon as it has completely cooled (W_2).
7. Weight of residue = $(W_2 - W_1)$ mg.
   W_2 and W_1 should be expressed in mg.

(b) Total fixed solids

1. Keep the same dish used for determining total residue in a muffle furnace for 1 hour at 550°C.
2. Allow the dish to partially cool in air until most of the heat has dissipated, then transfer to a desiccator for final cooling in a dry atmosphere.
3. Weigh the dish as soon as it has cooled (W_3).
4. Weight of total fixed residue = $(W_3 - W_1)$ mg.
   W_3 and W_1 should be expressed in mg.

(c) Total dissolved solids

1. Filter a measured portion of the mixed sample (50 or 100 mL) through a filter paper and collect the filtrate in a previously prepared and weighed evaporating dish.
2. Repeat the steps 3 to 6 outlined in total solids procedure.
3. Weight of dissolved solids = $(W_5 - W_4)$ mg.
   W_4 = Weight of empty evaporating dish in mg.
   W_5 = Weight of empty evaporating dish in mg + Residue left after evaporating the filtrate in mg.

(d) Total suspended solids = Total solids – Total dissolved solids.

(e) Total volatile solids = Total solids – Total fixed solids.

(f) Fixed dissolved solids

1. Keep the same evaporating dish used in determining total dissolved solids in a muffle furnace for 1 hour at 550°C.
2. Repeat the steps 2 and 3 outlined in total fixed solids procedure.
3. Weight of fixed dissolved solids = $(W_6 - W_4)$ mg.
   W_6 = Weight of empty evaporating dish + Fixed solids left after ignition at 550°C.

(g) Volatile dissolved solids = Total dissolved solids – Fixed dissolved solids.

(h) Fixed suspended solids = Total fixed solids – Fixed dissolved solids.

(i) Volatile suspended solids = Total volatile solids – Volatile dissolved solids.

(j) Settleable solids by volume

1. Fill an imhoff cone to the litre mark with a thoroughly mixed sample.
2. Settle for 45 minutes.
3. Gently stir the sides of the cone with a rod or by spinning.
4. Settle 15 minutes longer.
5. Record the volume of settleable matter in the cone as mL/L.

Observation

Sl. no.	*Item*	*Sample I*	*Sample II*	*Sample III*
1.	Volume of sample taken			
2.	Wt. of empty evaporating dish = W_1 mg (For total dissolved solids)			
3.	Wt. of dish + total solids = W_2 mg			
4.	Total solids = $(W_2 - W_1)$ mg			
5.	Wt. of dish + fixed solids = W_3 in mg			
6.	Fixed solids in mg = $(W_3 - W_1)$			

Contd...

7.	Wt. of empty evaporating dish = W_4 mg (For total dissolved solids)			
8.	Wt. of dish + total dissolved solids = W_5 mg			
9.	Total dissolved solids = ($W_5 - W_4$) mg			
10.	Wt. of dish + fixed dissolved solids = W_6 mg			
11.	Fixed dissolved solids = ($W_6 - W_4$) mg			
12.	Total solids in mg/L			
13.	Total fixed solids in mg/L			
14.	Total dissolved solids in mg/L			
15.	Total suspended solids in mg/L			
16.	Total volatile solids in mg/L			
17.	Fixed dissolved solids in mg/L			
18.	Volatile dissolved solids in mg/L			
19.	Fixed suspended solids in mg/L			
20.	Volatile suspended solids in mg/L			
21.	Settleable solids in mg/L			

Calculation

1. mg/L total solids $= \dfrac{\text{mg total solids} \times 1000}{\text{mL of sample}}$
2. mg/L total fixed solids $= \dfrac{\text{mg total fixed solids} \times 1000}{\text{mL of sample}}$
3. mg/L total dissolved solids $= \dfrac{\text{mg of total dissolved solids} \times 1000}{\text{mL of sample}}$
4. mg/L total suspended solids = mg/L of total solids – mg/L of total dissolved solids
5. mg/L total volatile solids = mg/L of total solids – mg/L of total fixed solids
6. mg/L fixed dissolved solids $= \dfrac{\text{mg fixed dissolved solids} \times 1000}{\text{mL of sample}}$
7. mg/L volatile dissolved solids = mg/L total dissolved solids – mg/L of fixed dissolved solids
8. mg/L fixed suspended solids = mg/L total fixed solids – mg/L fixed dissolved solids
9. mg/L volatile suspended solids= mg/L total volatile solids – mg/L volatile dissolved solids

Note: These calculations need to be shown only for one sample.

Results

Sl. no.	*Items*	*Sample I*	*Sample II*	*Sample III*
1.	mg/L of total solids			
2.	mg/L of total fixed solids			
3.	mg/L of total dissolved solids			
4.	mg/L of total suspended solids			
5.	mg/L of total volatile solids			
6.	mg/L of fixed dissolved solids			
7.	mg/L of volatile dissolved solids			
8.	mg/L of fixed suspended solids			
9.	mg/L of volatile suspended solids			
10.	mg/L of settleable solids			

Discussion

Questions

1. What is the application of determination of settleable solids?
2. Explain the significance of determination of total solids in sanitary engineering.
3. How will the volatile solids affect the strength of sewage? Why?
4. Why do you determine the fixed solids by igniting at 600°C? How will the result be affected, if it has magnesium carbonate content?
5. What significant information is furnished by the determination of volatile solids?
6. What is sludge volume index?

Experiment No. _____ **Date** _____

TURBIDITY

Aim

To determine the turbidity of the given sample using Nephelometer in NTU.

Principle

The method presented below is based on a comparison of the intensity of light scattered by the sample in specific conditions with the intensity of light scattered by standard reference suspension under the same condition. The higher the intensity of scattered lights, higher the turbidity. Formazine polymer, which has gained acceptance as the turbidity standard reference suspension is used as a reference turbidity standard suspension for water. It is easy to prepare and is more reproducible in its lights scattering properties than the clay or turbid natural water standards previously used. The turbidity of a given concentration of formazine has an approximate turbidity of 100 NTU, when measured on candle turbidity meter. Nephelometric turbidity units based on formazine preparation will have approximate units derived from Jackson candle turbidimeter but will not be identical to them.

Apparatus

Nephelometer with accessories

Reagents

(i) Turbidity free distilled water (for setting zero).
(ii) Formazine turbidity concentrate (hydrazine sulphate + hexamine).
(iii) Formazine standard (for setting 100 of the instrument).

Preparation of Turbidity Free Distilled Water

Pass distilled water through a membrane filter having a precision pore size of less than 10 microns (Whatman filter No. 42). Rinse collecting flask atleast twice with such filtered water and discard the next 200 mL. Use this filtered water for setting zero of the instrument.

Preparation of Formazine Turbidity Concentrate

(a) Solution I

Weigh accurately 5 g of 'Anal–R' quality hydrazine sulphate $(NH_2)_2H_2SO_4$ into a 500 mL volumetric flask and add distilled water to make up to the mark. Leave the mixture to stand for 4 hours.

(b) Solution II

Weigh accurately 50g of 'Anal–R' quality hexamethylene tetramine $(CH_2)_6N_4$ (hexamine) into a 500 mL volumetric flask and add distilled water to make up to the mark.

Mix equal volume of solution I and II to form formazine turbidity concentrate. Allow it to stand in a closed container at 25°C to 30°C for 48 hours to produce insoluble white turbidity corresponding to 4000 NTU.

Note: Once prepared, formazine turbidity concentrate (which corresponds to 10000 ppm SiO_2) is stable for 2 to 3 months.

Preparation of Formazine Standard

Dilute 25mL of the formazine turbidity concentrate to 1 litre with turbidity free distilled water to obtain 250 ppm or 100 NTU for setting '100' of the instrument.

Note: Formazine standard 100 NTU should be prepared weekly.

Procedure

(1) Switch the instrument on.
(2) Open the lid of the sample compartment.
(3) Insert a test tube filled with distilled water into the sample compartment. Close the lid.
(4) Adjust 'SET 0' control to get '0' displayed on the read out.
(5) Open the lid. Replace the test tube filled with distilled water with a test tube filled with formazine standard. Close the lid.
(6) Adjust the 'SET 100' control to get '100' displayed on the read out.
(7) Repeat the above operation to get consistent values of 0 to 100 within 1% to 2%.

Measurement of turbidity less than 100 NTU

1. Thoroughly shake the sample.
2. Wait until air bubbles disappear and pour the sample into the nephelometer tube.
3. Read the turbidity directly from the instrument.

Measurement of turbidity above 100 NTU

Dilute the sample with one or more volume of turbidity free distilled water until the turbidity fall below 100 NTU.

$$\text{NTU of sample} = \frac{A(B + C)}{C}$$

where,

A = NTU found in diluted sample

B = volume of dilution water in mL

C = sample volume taken for dilution in mL

Observation

0 –100 NTU		*>100 NTU*			
Sample no.	***NTU***	***A mL***	***B mL***	***C mL***	***NTU = A(B+C)/C***

Results

Description of sample	*Turbidity in NTU*

Discussion

Questions

1. Where do you find the adverse effects of turbidity in environmental engineering? Mention two instances.
2. Discuss the significance of determination of turbidity in sanitary engineering.
3. Discuss the nature of materials causing turbidity in
   (a) River water during flash flood
   (b) Polluted river water
   (c) Domestic wastewater
4. What is the standard unit of turbidity?
5. What are NTU and JTU?

Experiment No. _____ **Date** _____

DETERMINATION OF ALKALINITY

Aim

To determine the amount of the following types of alkalinity present in the given samples:

(a) Hydroxide alkalinity
(b) Carbonate alkalinity
(c) Bicarbonate alkalinity
(d) Hydroxide–Carbonate alkalinity
(e) Carbonate–Bicarbonate alkalinity

Principle

The alkalinity of water is a measure of its capacity to neutralize acids. It is primarily due to salts of weak acids, although weak or strong bases may also contribute. Alkalinity is usually imparted by bicarbonate, carbonate and hydroxide. It is measured volumetrically by titration with 0.02 N sulphuric acid and is reported in terms of $CaCO_3$ equivalent. For samples whose initial pH is above 8.3, the titration is conducted in two steps. In the first step, the titration is conducted until the pH is lowered to 8.2, the point at which phenolphthalein indicator turns from pink to colourless. This value corresponds to the points for conversion of carbonate to bicarbonate ion. The second phase of titration is conducted until the pH is lowered to 4.5, corresponds to methyl orange end point, which corresponds to the equivalence points for the conversion of bicarbonate ion to carbonic acid.

Apparatus

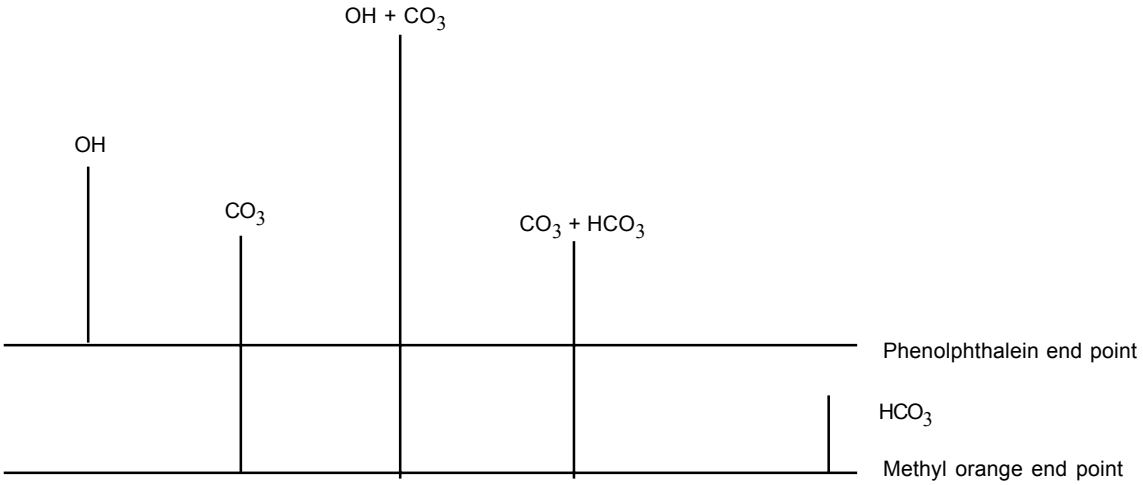
1. Burette 2. Erlenmeyer flask 3. Pipettes

Reagents

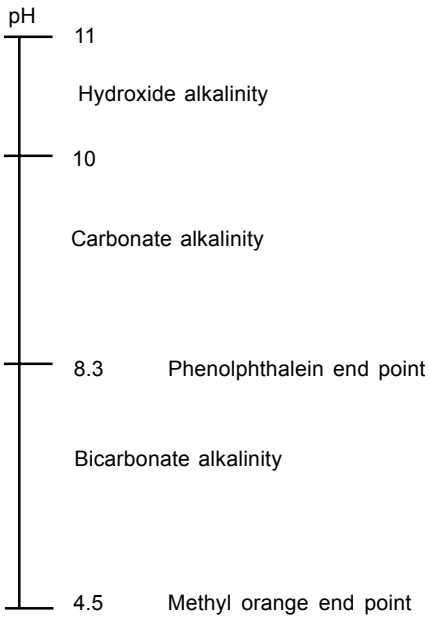
1. Carbon dioxide free distilled water.
2. Phenolphthalein indicator.
3. Methyl orange indicator.
4. 0.1 N sodium thiosulphate solution
5. 0.02 N sulphuric acid.

Procedure

1. Pipette 50 mL of sample into a clean Erlenmeyer flask (V).
2. Add one drop of sodium thiosulphate solution, if residual chlorine is present.
3. Add two drops of phenolphthalein indicator; if the pH is above 8.3, colour of solution becomes pink.
4. Titrate against standard sulphuric acid in the burette, till the colour just disappears. Note down the volume (V_1).
5. Then add two drops of methyl orange indicator, the colour turns yellow.
6. Again titrate against acid, until the colour turns to orange yellow. Note down the total volume (V_2).



Graphical Representation of Titration of Samples Containing Various Forms of Alkalinity



Observation

0.02 N H_2SO_4 × sample (Methyl orange/phenolphthalein indicator)

Description of sample	*Trial no.*	*Burette reading (phenolphthalein indicator)*		*Volume of acid used V_1*	*Burette reading (methyl orange indicator)*		*Volume of acid used V_2*
		Initial	*Final*		*Initial*	*Final*	

Calculation

1. Phenolphthalein alkalinity (P) as mg/L $CaCO_3 = \frac{V_1 \times 1000}{\text{mL of sample}}$

2. Total alkalinity (T) as mg/L $CaCO_3 = \frac{V_2 \times 1000}{\text{mL of sample}}$

The types of alkalinities present in the samples are calculated using the equations given in the following table and the results are tabulated.

Result of titration	*Hydroxide alkalinity as $CaCO_3$*	*Carbonate alkalinity as $CaCO_3$*	*Bicarbonate alkalinity as $CaCO_3$*
P = O	O	O	T
P < ½T	O	2P	T – 2P
P = ½T	O	2P	O
P > ½T	2P – T	2 (T – P)	O
P = T	T	O	O

Results

Description of sample	*Hydroxide alkalinity as $CaCO_3$ in mg/L*	*Carbonate alkalinity as $CaCO_3$ in mg/L*	*Bicarbonate alkalinity as $CaCO_3$ in mg/L*	*Hydroxide carbonate alkalinity as $CaCO_3$ in mg/l*	*Carbonate bicarbonate alkalinity as $CaCO_3$ in mg/L*

Discussion

Questions

1. Which is the major form of alkalinity? How is it formed?
2. What is excess alkalinity? How do you express it?
3. Why do we take 0.02 N H_2SO_4 for the titration?
4. The water where algae are flourishing is alkaline. Why? Will there be diurnal variation in pH?
5. Why does the pH change on aerating the water?
6. For efficient coagulation the water must be alkaline. Why?
7. Why do we use CO_2 free distilled water for analysis?

Experiment No. _____ **Date** _____

DETERMINATION OF HARDNESS

Aim

To determine the total hardness of the given samples by EDTA titrimetric method.

Principle

Originally, the hardness of water was understood to be a measure of the capacity of water for precipitating soap. Soap is precipitated chiefly by the calcium and magnesium ions commonly present in water, but may also be precipitated by ions of other polyvalent metals, such as aluminium, iron, manganese, strontium and zinc, and by hydrogen ions. Because, all but the first two are usually present in insignificant concentrations in natural waters, hardness is defined as a characteristic of water, which represents the total concentration of just the calcium and the magnesium ions expressed as calcium carbonate. However, if present in significant amounts, other hardness producing metallic ions should be included.

When the hardness is numerically greater than the sum of the carbonate alkalinity and the bicarbonate alkalinity, the amount of hardness, which is equivalent to the total alkalinity, is called *carbonate hardness*; the amount of hardness in excess of this is called *non-carbonate hardness*. When the hardness is numerically equal to or less than the sum of carbonate and bicarbonate alkalinity all of the hardness is carbonate hardness and there is no non-carbonate hardness. The hardness may range from zero to hundreds of milligrams per litre in terms of calcium carbonate, depending on the source and treatment to which the water has been subjected.

Ethylenediamine tetra-acetic acid and its sodium salts (EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as *Eriochrome black T* is added to an aqueous solution containing calcium and magnesium ions at a pH of 10 ± 0.1, the solution will become wine red. If EDTA is then added as a titrant, the calcium and magnesium will be complexed. After sufficient EDTA has been added to complex all the magnesium and calcium, the solution will turn from wine red to blue. This is the end point of the titration.

Apparatus

1. Burette
2. Pipette
3. Erlenmeyer flask
4. Bottle etc.

Reagents

1. Standard EDTA titrant (0.01 M)
2. Eriochrome black T indicator
3. Ammonia buffer solution

Procedure

1. Dilute 25 mL of sample (V) to about 50 mL with distilled water in an Erlenmeyer flask.
2. Add 1 mL of buffer solution.
3. Add two drops of indicator solution. The solution turns wine red in colour.
4. Add the standard EDTA titrant slowly with continuous stirring until the last reddish tinge disappears from the solution. The colour of the solution at the end point is blue under normal conditions.
5. Note down the volume of EDTA added (V_1).

Observation

Sample no.	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of EDTA (mL)*
			Initial	*Final*	

Calculation

$$\text{Hardness as } CaCO_3 = \frac{V_1 \times S \times 1000}{V} \text{ mg/L}$$

where, S = mg $CaCO_3$ equivalent to 1 mL of EDTA titrant

= 1 mg $CaCO_3$

$$\text{Hardness as } CaCO_3 = \frac{1000\ V_1}{V} = \ldots\ldots \text{ mg/L}$$

Results

Sample no. or description	*Total hardness in mg/L as $CaCO_3$*

Discussion

Questions

1. What is degree of hardness? How will you classify water in terms of degree of hardness?
2. What is pseudo-hardness?
3. Explain the significance of determination of hardness of water in environmental engineering.
4. How can you remove permanent hardness from water?
5. Can you determine temporary hardness and permanent hardness separately? If yes, how?
6. What are the principal cations causing hardness in water and the major anions associated with them?
7. How is hardness classified?
8. Why is softening of water necessary? What are the advantages of soft water?

Experiment No. _____ **Date** _____

DETERMINATION OF pH

Aim

To determine the pH of given samples using (1) universal indicator (2) pH paper, and (3) digital pH meter.

Principle

pH value of water indicates the hydrogen ion concentration in water and concept of pH was put forward by Sorenson (1909). pH is expressed as the logarithm of the reciprocal of the hydrogen ion concentration in moles/litre at a given temperature. The pH scale extends from 0 (very acidic) to 14 (very alkaline) with 7 corresponding to exact neutrality at 25°C. pH is used in the calculation of carbonate, bicarbonate and CO_2, corrosion and stability index etc. While the alkalinity or acidity measures the total resistance to the pH change or buffering capacity, the pH gives the hydrogen ion activity. pH can be measured colorimetrically or electrometrically. Colorimetric method is used only for rough estimation. It can be done either by using universal indicator or by using pH paper. The hydrogen electrode is the absolute standard for the measurement of pH. They range from portable battery operated units to highly precise instruments. But glass electrode is less subjected to interferences and is used in combination with a calomel reference electrode. This system is based on the fact that a change of 1 pH unit produces an electric charge of 59.1 mV at 25°C.

Apparatus

1. pH meter with electrode
2. Beaker
3. Thermometer
4. Colour comparator with discs
5. Cuvettes

Reagents

1. Buffer solutions
2. pH paper
3. Universal indicator

Procedure

(a) Using Universal Indicator

1. 10 mL of sample is taken in a cuvette.

2. Another 10 mL sample is taken in another cuvette and 0.2 mL of universal indicator is added and placed in the hole provided for.
3. A colour disc corresponding to this indicator is inserted into the comparator and the disc rotated such that the 2 circles indicate identical colours.
4. The reading is noted.
5. The procedure can be repeated using an indicator whose range is near the value obtained.
6. The exact pH is obtained.

   (If comparators are not available, compare the colour with colours given in the chart.)

(b) Using pH Papers

1. Dip the pH paper in the sample.
2. Compare the colour with that of the colour given on the wrapper of the pH paper book.
3. Note down the pH of the sample along with its temperature.

(c) Using pH Meter

1. Follow the manufacturer's operating instructions.
2. Dip the electrode in the buffer solution of known pH.
3. Switch on the power supply and take the reading. Standardize the instrument using the calibrating knob.
4. After cleaning, again dip the electrodes in the buffer solution of pH 7. Note the reading. If it is 7, the instrument is calibrated. If not, correct the value and is manipulated so that the reading in the dial comes to 7.0.
5. A solution whose pH is to be found is taken in a beaker and the temperature knob is adjusted such that the temperature of solution is same as that in dial.
6. The electrode is washed with distilled water and reused with the solution and then it is dipped in the solution.
7. The reading on the dial indicates the pH of the solution.

Results

Sample no.	*pH*		
	pH paper	*pH meter*	*Universal indicator*
1			
2			
3			

Discussion

Questions

1. Discuss the relationship between (a) pH and hydrogen ion concentration (b) pH and hydroxide ion concentration?
2. A decrease in pH of 1 unit represents how much of an increase in hydrogen ion concentration?
3. Why is it necessary to maintain the pH of water nearly 7?
4. What is a buffer solution? Give examples.

Experiment No. _____ **Date** _____

DETERMINATION OF CHLORIDE

Aim

To determine the amount of chloride (in the form of Cl^-) present in the given water sample by Mohr's method.

Principle

If water containing chlorides is titrated with silver nitrate solution, chlorides are precipitated as white silver chloride. Potassium chromate is used as indicator, which supplies chromate ions. As the concentration of chloride ions approaches extinction, silver ion concentration increases to a level at which reddish brown precipitate of silver chromate is formed indicating the end point.

Apparatus

1. Burette
2. Pipettes
3. Erlenmeyer flasks
4. Measuring cylinder

Reagents

1. Chloride free distilled water.
2. Standard silver nitrate solution (0.0141N)
3. Potassium chromate indicator.
4. Acid or alkali for adjusting pH.

Procedure

1. Take 50mL of sample (V) and dilute to 100mL.
2. If the sample is coloured add 3mL of aluminium hydroxide, shake well; allow to settle, filter, wash and collect filtrate.
3. Sample is brought to pH 7–8 by adding acid or alkali as required.
4. Add 1mL of indicator (Potassium chromate).

5. Titrate the solution against standard silver nitrate solution until a reddish brown precipitate is obtained. Note down the volume (V_1).
6. Repeat the procedure for blank and note down the volume (V_2).

Observation

Water sample vs Silver nitrate (0.0141 N) (Potassium chromate indicator)						
Sample no.	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of silver nitrate (mL)*	*Chloride mg/L*
			Initial	*Final*		
1	1					
	2					
	3					
2	1					
	2					
	3					
3	1					
	2					
	3					
Distilled Water	1					
	2					
	3					

(Specimen calculation:) for one sample (Sample No. …...)

V =

V_1 =

V_2 =

N =

$$\text{Chloride in mg/L} = \frac{(V_1 - V_2) \times N \times 35.46 \times 1000}{V}$$

$$= \frac{(V_1 - V_2) \times 500}{V} = \text{..... mg / L}$$

Results

Description of sample	*Chloride concentration in mg/L*

Discussion

Questions

1. Explain the significance of high chloride in water.
2. What are the sources of chloride in water?
3. Explain the need for blank correction.
4. Why must be the sample pH neither high nor low?
5. Why the normality of silver nitrate solution is taken as zero?
6. Would the analytical result by Mohr's method for chlorides be higher, lower, or the same as the true value if an excess indicator were accidentally added to the sample? Why?
7. What are the methods of determination of chlorides?
8. Why do the water has lower content of salt than sewage?

Experiment No. _____ **Date** _____

DETERMINATION OF IRON AND MANGANESE

A. DETERMINATION OF IRON

Aim

To determine the quantity of iron present in the given sample of water.

Principle

Iron is usually present in natural water and is not objectionable, if concentration is less than 0.3 ppm. It may be in true solution in colloidal state that may be peptized by organic matter, in the inorganic and organic iron complexes, or in relatively coarse suspended particles. It may be either ferrous or ferric, suspended or filterable. Iron exists in soils and minerals mainly as insoluble ferric oxide and iron sulphide (pyrite). It occurs in some areas, also as ferrous carbonate (siderite), which is very slightly soluble.

The phenanthroline method is the preferred standard procedure for the measurement of iron in water except when phosphate or heavy metal interferences are present. The method depends upon the fact that 1, 10-phenanthroline combine with Fe^{++} to form an orange-red complex. Its colour conforms to Beer's law and is readily measured by visual or photometric comparison. Small concentration of iron can be most satisfactorily determined by colorimetric analysis. It is also based on Beer's law. By measuring the intensities of transmitted and incident light through a coloured solution and knowing its optical density or transmission, we can prepare a calibration curve and subsequent concentration can be read.

Phenanthroline Method

Apparatus

1. Colorimetric equipment; one of the following is required:
   (*a*) Spectrophotometer, for use at 510 nm, providing a light path of 1 cm or longer.
   (*b*) Nessler tubes, matched, 100 mL, tall form.
2. Glassware like conical flasks, pipettes and glass beads.

Reagents

1. Hydrochloric acid
2. Hydroxylamine solution
3. Ammonium acetate buffer solution
4. Sodium acetate solution
5. Phenanthroline solution
6. Stock iron solution
7. Standard iron solution (1 mL = 10 μg Fe)

Procedure

1. Pipette 10, 20, 30 and 50 mL. Standard iron solution into 100 mL conical flasks.
2. Add 1 mL hydroxylamine solution and 1 mL sodium acetate solution to each flask.
3. Dilute each to about 75 mL with distilled water.
4. Add 10 mL phenanthroline solution to each flask.
5. Make up the contents of each flask exactly to 100mL by adding distilled water and left stand for 10 minutes.
6. Take 50 mL distilled water in another conical flask.
7. Repeat steps 2 to 5 described above.
8. Measure the absorbance of each solution in a spectrophotometer at 508 nm against the reference blank prepared by treating distilled water as described in steps 6 and 7. Prepare a calibration graph taking meter reading on y-axis and concentration of iron on x-axis.
9. For visual comparison, pour the solution in 100 mL tall form Nessler tubes and keep them in a stand.
10. Mix the sample thoroughly and measure 50 mL into a conical flask.
11. Add 2 mL conc. hydrochloric acid (HCl) and 1mL hydroxylamine solution. Add a few glass beads and heat to boiling. To ensure dissolution of all the iron, continue boiling until the volume is reduced to 15 to 20 mL.
12. Cool the flask to room temperature and transfer the solution to a 100 mL Nessler tube.
13. Add 10 mL ammonium acetate buffer solution and 2 mL phenanthroline solution and dilute to the 100 mL mark with distilled water.
14. Mix thoroughly and allow at least 10 to 15 minutes for maximum colour development.
15. Measure the absorbance of the solution in a 1cm cell in a spectrophotometer at 508 nm.
16. Read off the conc. of iron (mg Fe) from the calibration graph for the corresponding meter reading.
17. For visual comparison, match the colour of the sample with that of the standard prepared in steps 1 to 7 above.
18. The matching colour standard will give the concentration of iron in the sample (μg Fe).

Observation

Standard iron solution in mL	*Iron content in µg*	*Absorbance*

Sample no.	*Absorbance*	*Iron content from graph in µg*	*Iron as Fe in mg/L*

Sample calculation

iron (Fe) in mg/L = µg Fe/mL of sample

= ..... mg/L

Results

Sample no. or description	*Iron content in mg/L (Fe)*

Discussion

B. DETERMINATION OF MANGANESE

Aim

To determine the quantity of manganese present in the given sample.

Principle

Manganese exists in the soil principally as manganese dioxide, which is very insoluble in water containing carbon dioxide. Under anaerobic conditions, the manganese in the dioxide form is reduced from an oxidation state of IV to II and solution occurs. The manganese in ground water is of divalent form. It may be present in trivalent or quadrivalent form also.

The concentration of manganese seldom exceeds a few mg/L. So colorimetric methods are applicable. The methods are (1) persulphate method, and (2) periodate method. Both methods depend upon oxidation of manganese from its lower oxidation state to VII where it forms the highly coloured permanganate ion. The colour produced is directly proportional to the concentration of manganese present over a considerable range of concentration in accordance with Beer's law. So it can be measured by eye or photometric means. Provisions must be made to overcome the influence of chlorides. Manganese can also be determined by atomic absorption spectrophotometry. Persulphate method is suitable because pretreatment of samples is not required. Chloride concentration is reduced by using mercuric sulphate. Persulphate oxidises manganese to permanganate in the presence of silver nitrate as catalyst. The colour intensity is observed at a wavelength of 525 nm in a spectrophotometer.

Periodate method is used when concentrations are below 0.1 mg/L. To obtain complete oxidation of small amounts of manganese, silver nitrate is added and the heating time is increased.

Apparatus

1. Colorimetric equipment: one of the following is required:
   (a) Spectrophotometer, for use at 252 nm, providing a light path of 1 cm or longer.
   (b) Nessler tubes, matcheds, 100 mL tall form.
2. Glassware like conical flasks, measuring cylinder and pipette.

Reagents

1. Special reagent
2. Ammonium persulphate
3. Standard manganese solution
4. Hydrogen peroxide 30%.

Procedure

1. Take 50 mL of the sample in a conical flask. Add 50 mL distilled water to it.
2. Pipette 1, 2, 3, 4, and 8 mL of standard manganese solution to different flasks, and dilute each to 100 mL using distilled water.
3. Add 5 mL special reagent to all the flasks.
4. Concentrate the solutions in all the flasks to about 90 mL boiling.
5. Add 1 g ammonium persulphate to all the flasks, bring to boiling and boil for 1 minute.
6. Remove all the flasks from the heat source and let stand for 1 minute.

7. Then cool the flasks under the tap water.
8. Dilute the contents in all the flasks to 100 mL with distilled water and mix. Pour the contents into 100 mL Nessler tubes.
9. Match the colour of the sample with that of the colour standards. Note down the concentration of Mn in µg.
10. If the spectrophotometer is used, one distilled water blank has to be prepared along with the colour standards.
11. Measure the absorbance of each solution in a 1 cm cell at 525 nm against the reference blank prepared by treating distilled water.
12. Prepare the calibration graph taking meter reading along y-axis and concentration of manganese (in µg) in colour standards on x-axis.
13. Keep the sample in the spectrophotometer and note down the meter reading.
14. Read off from the graph, the corresponding concentration of manganese in µg.

Observation

Concentration of Mn in colour standards in µg	*Spectrophotometer reading*

Sample no. or description	*Volume of sample taken*	*Concentration of Mn in sample in µg of matching colour standard or from the graph*	*Mg/L of Mn*

Sample calculation

$$\text{Mn in mg/L} = \frac{\mu\text{g of Mn}}{\text{mL sample}}$$

Results

Sample no. or description	*Concentration of Mn in mg/L*

Discussion

Questions

1. How are the iron and manganese removed from water?
2. Explain the significance of Fe and Mn in environmental engineering.
3. In what oxidation state must the manganese be for colorimetric measurement?

Experiment No. _____ **Date** _____

DETERMINATION OF SULPHATE AND SULPHIDE

A. DETERMINATION OF SULPHATE

Aim

To determine the amount of sulphate present in the given samples.

Principle

Sulphate is widely distributed in nature and may be present in natural water in concentrations ranging from a few to several thousand milligrams/litre. Sulphates are of considerable concern because they are indirectly responsible for two serious problems often associated with the handling and treatment of wastewater. Odour and sewer corrosion problem result from the reduction of sulphates to hydrogen sulphide under anaerobic conditions.

Sulphates can be determined by

1. Gravimetric method with ignition of residue.
2. Gravimetric method with drying of residue.
3. Turbidimetric method.

1. Gravimetric Method with Ignition of Residue

Principle

Sulphate is precipitated in hydrochloric acid medium as barium sulphates by the addition of barium chloride. The precipitation is carried out near the boiling temperature and after a period of digestion the precipitate is filtered; washed with water until free of chlorides, ignited and weighed as barium sulphates.

Apparatus

1. Drying oven
2. Desiccator
3. Steam bath
4. Analytical balance
5. Ashless filter paper (Whatman filter paper No. 42)

6. Muffle furnace
7. Glassware like funnel, flask and pipette

Reagents

1. Methyl red indicator solution
2. Hydrochloric acid
3. Barium chloride solution. Gravimetric method with drying of residue.
4. Silver nitrate–nitric acid reagent

Procedure

1. Take 250 mL of the sample in a conical flask.
2. Adjust the acidity with HCl to 4.5 to 5 using a pH meter or the orange colour of methyl red indicator.
3. Then add an additional 1 to 2 mL HCl.
4. Heat the solution to boiling and while stirring gently, add barium chloride solution slowly until precipitation appear to be completed. Then add about 2 mL in excess.
5. Digest the precipitate at 80°C to 90°C preferably overnight but for not less than 2 hours.
6. Filter the contents in the flask through an ashless filter paper.
7. Wash the precipitate with small portion of warm distilled water until the washing is free of chloride as indicated by testing with silver nitrate–nitric acid reagent.
8. Place the precipitate along with filter paper in a crucible after finding its empty weight and dry it.
9. Keep the crucible in a muffle furnace and ignite at 800°C for 1 hour.
10. Cool in a desiccator and weigh.
11. Find weight of the barium sulphate precipitate.

2. Gravimetric Method with Drying of Residue

If organic matter is not present in the sample, first method can be done without igniting and instead drying the residue and weighing.

3. Turbidimetric Method

Principle

The turbidimetric method of measuring sulphate is based upon the fact that barium sulphate tends to precipitate in a colloidal form and that this tendency is enhanced in presence of a sodium chloride—hydrochloric acid solution containing glycerol and other organic compounds. The absorbance of the barium sulphate solution is measured by a nephelometer or turbidimeter and the sulphate iron concentration, determined by comparison of the reading with a standard curve.

Apparatus

1. Nephelometer or Turbidimeter
2. Magnetic stirrer

3. Stopwatch
4. Measuring spoon 0.2 to 0.3 mL capacity.

Reagents

1. Conditioning agent
2. Barium chloride
3. Standard sulphate solution

Procedure

1. Measure 100 mL or suitable portion of the sample into a 250 mL Erlenmeyer flask.
2. Add 5 mL of conditioning reagent and mix it by placing on a magnetic stirrer.
3. Add a spoonful of barium chloride crystals and begin timing immediately.
4. Stir at constant speed exactly for one minute.
5. After stirring pour some of the solution into the absorption cell of the photometer, and measure the turbidity at 30 second intervals for four minutes.
6. Usually maximum turbidity occurs within two minutes and the reading remains constant thereafter for 3 to 10 minutes. So, take reading with maximum turbidity occurring in within four minutes.
7. Prepare a calibration curve. The standards are prepared at 5 mg/L increments in the 0–40 mg/L sulphate range and their turbidity or absorbance read.
8. Absorbance versus sulphate concentration is plotted and a curve is obtained.
9. Finding the absorbance for a given sample, the concentration of sulphates in the solution is determined with the help of calibration curve.

Observation

Sample no. or description	*Volume of the sample (mL)*	*Empty weight of the crucible + filter paper*	*Wt. of crucible + residue after ignition + filter paper*	*Wt. of $BaSO_4$ precipitated*	*mg/L SO_4*

Weight of filter paper =.....

Calculation

$$SO_4 \text{ in mg/L} = \frac{\text{mg of } BaSO_4}{\text{mL of sample}} \times 411.6 = ...$$

Results

Sample no. or description	*mg/L of SO_4*

Discussion

B. DETERMINATION OF SULPHIDE

Aim

To determine the amount of sulphide present in the sample by titrimetric method.

Principle

Sulphides often occur in ground water especially in hot springs, in wastewater and polluted waters. Hydrogen sulphide escaping into the air from sulphide containing wastewater causes odour nuisance. It is highly toxic and cause corrosion of sewers and pipes. Sulphides include H_2S and HS^- and acid soluble metallic sulphides present in the suspended matter.

Iodine reacts with sulphide in acid solution, oxidising it to sulphur; a titration based on this reaction is an accurate method for determining sulphides at concentration above 1mg/L if interferences are absent and if loss of H_2S is avoided.

Apparatus

1. Burette
2. Pipette
3. Erlenmeyer flask.

Reagents

1. Hydrochloric acid
2. Standard iodine solution (0.025N)
3. Standard sodium thiosulphate solution (0.025N)
4. Starch solution

Procedure

1. Measure from a burette 10mL of iodine into a 500 mL flask.
2. Add distilled water and bring the volume to 20 mL.
3. Add 2 mL of 6 N HCl.
4. Pipette 200 mL sample into the flask, discharging the sample under the surface of solution.
5. If the iodine colour disappears, add more iodine so that the colour remains.
6. Titrate with sodium thiosulphate solution, adding a few drops of starch solution, as the end point is approached and continuing until the blue colour disappears.

Observation

Sample no. or description	*Volume of iodine solution used (a mL)*	*Volume of sodium thiosulphate solution used (b mL)*	*Volume of sample used*	*mg/L sulphide*

Calculation

$$\text{mg/L sulphide} = \frac{400\ (a - b)}{\text{mL of sample}}$$

where, a = mL 0.025 N iodine used

b = mL 0.025 N sodium thiosulphate solution used.

Results

Sample no. or description	*mg/L (Sulphide) in the sample*

Discussion

Questions

1. What is the significance of high sulphate concentration in water supplies and in wastewater disposal?
2. What is the purpose of digestion of the sample in the gravimetric analysis for sulphates?
3. Explain the significance of the determination of sulphide concentration in environmental engineering.
4. The water to be used for the preparation of cement concrete products should be free from excess of sulphates and chlorides. Why?

Experiment No. _____ **Date** _____

JAR TEST FOR DETERMINING OPTIMUM COAGULANT DOSAGE

Aim

To determine the optimum coagulant dosage for clarifying the given sample of water by using alum as the coagulant and performing the jar test experiment.

Principle

Coagulants are used in water treatment plants

(i) to remove natural suspended and colloidal matter,

(ii) to remove material which do not settle in plain sedimentation, and

(iii) to assist in filtration.

Alum [$Al_2(SO_4)_3$. $18H_2O$] is the most widely used coagulant. When alum solution is added to water, the molecules dissociate to yield SO_4^{2-} and Al^{3+}. The +ve species combine with negatively charged colloidal to neutralise part of the charge on the colloidal particle. Thus, agglomeration takes place. Coagulation is a quite complex phenomenon and the coagulant should be distributed uniformly throughout the solution. A flash mix accomplishes this.

Jar test is simple device used to determine this optimum coagulant dose required. The jar test, device consists of a number of stirrers (4 to 6) provided with paddles. The paddles can be rotated with varying speed with the help of a motor and regulator. Samples will be taken in jars or beakers and varying dose of coagulant will be added simultaneously to all the jars. The paddles will be rotated at 100 rpm for 1 minute and at 40 rpm for 20 to 30 minutes, corresponding to the flash mixing and slow mixing in the flocculator of the treatment plant. After 30 minutes settling, supernatant will be taken carefully from all the jars to measure turbidity. The dose, which gives the least turbidity, is taken as the optimum coagulant dose.

Apparatus

1. Jar test apparatus
2. Glass beakers
3. Pipette
4. Nephelometer
5. pH meter

Reagents

1. Alum solution (1mL containing 10 mg of alum)
2. Lime
3. Acid/alkali

Procedure

1. Take 1-litre beakers and fill them with sample up to the mark.
2. Keep each beaker below each paddle and lower the paddles, such that each one is about 1cm above the bottom.
3. Find the pH of the sample and adjust it to 6 to 8.5.
4. Pipette 1, 2, 3, 4, 5, 6 mL of the alum solution into the test samples.
5. Immediately run the paddles at 100 rpm for 1 minute.
6. Reduce the speed to 30–40 rpm and run at this rate for 30 minutes.
7. Stop the machine, lift out the paddles and allow to settle for 30 minutes.
8. Find the residual turbidity of the supernatant using nephelometer.
9. Plot a graph with alum dosage along x-axis and turbidity along y-axis.
10. The dosage of alum, which represents least turbidity, gives Optimum Coagulant Dosage (O.C.D.).
11. Repeat steps 1–10 with higher dose of alum, if necessary.

Observation

Trial no.	*Alum dosage in mg/L*	*Turbidity in NTU*

Results

Optimum coagulant dosage = ...

Discussion

Questions

1. Why is alum preferred to other coagulants?
2. What is the difference between coagulation and flocculation?
3. What are coagulant aids?
4. Write the significance of pH in coagulation using alum.
5. What factors affect the sedimentation of a discrete particle setting in a quiescent liquid?

Experiment No. _____ **Date** _____

DETERMINATION OF DISSOLVED OXYGEN

Aim

The aim of the experiment is to determine the quantity of dissolved oxygen present in the given sample(s) by using modified Winkler's (Azide modification) method.

Principle

Dissolved Oxygen (D.O.) levels in natural and wastewaters are dependent on the physical, chemical and biochemical activities prevailing in the water body. The analysis of D.O. is a key test in water pollution control activities and waste treatment process control.

Improved by various techniques and equipment and aided by instrumentation, the Winkler (or iodometric) test remains the most precise and reliable titrimetric procedure for D.O. analysis. The test is based on the addition of divalent manganese solution, followed by strong alkali to the water sample in a glass-stoppered bottle. D.O. present in the sample rapidly oxidises in equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions and upon acidification, the oxidised manganese reverts to the divalent state, with the liberation of iodine equivalent to the original D.O. content in the sample. The iodine is then titrated with a standard solution of thiosulphate.

Apparatus

1. 300 mL capacity bottle with stopper
2. Burette
3. Pipettes, etc.

Reagents

1. Manganous sulphate solution ($MnSO_4.4H_2O$)
2. Alkali-iodide azide reagent
3. Conc. sulphuric acid (36 N)
4. Starch indicator
5. Standard sodium thiosulphate solution (0.025 N)
6. Standard potassium dichromate solution (0.025 N)

Procedure

1. Add 2 mL of manganous sulphate solution and 2 mL of alkali-iodide azide reagent to the 300 mL sample taken in the bottle, well below the surface of the liquid.

   (The pipette should be dipped inside the sample while adding the above two reagents.)
2. Stopper with care to exclude air bubbles and mix by inverting the bottle at least 15 times.
3. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again.
4. After 2 minutes of settling, carefully remove the stopper, immediately add 3 mL concentrated sulphuric acid by allowing the acid to run down the neck of the bottle.
5. Restopper and mix by gentle inversion until dissolution is complete.
6. Measure out 203 mL of the solution from the bottle to an Erlenmeyer flask. As 2 mL each of manganese sulphate and azide reagent have been added, the proportionate quantity of yellow solution corresponds to 200 mL of sample is

   $$= \frac{200 \times 300}{300 - 4} = 203 \text{ mL}$$
7. Titrate with 0.025 N sodium thiosulphate solution to a pale straw colour.
8. Add 1–2 mL starch solution and continue the titration to the first disappearance of the blue colour and note down the volume of sodium thiosulphate solution added (V), which gives directly the D.O. in mg/L.

Observation

Sample × Standard sodium thiosulphate solution (0.025 N) (Starch indicator)

Description of sample	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of titrant mL*	*D.O. in mg/L*
			Initial	*Final*		
Sample I						
Sample II						
Sample III						

Results

Description of sample	*D.O. mg/L*
Sample I	
Sample II	
Sample III	

Discussion

Questions

1. Discuss the environmental significance of dissolved oxygen.
2. Most of the critical conditions related to dissolved oxygen deficiency occur during summer months. Why?
3. Why do we use 0.025 N sodium thiosulphate solution for the titration?
4. The turbulence of water should be encouraged. Why?
5. Draw the oxygen saturation curve.

Experiment No. _____ **Date** _____

DETERMINATION OF B.O.D. OF WASTEWATER SAMPLE

Aim

To determine the amount of B.O.D. exerted by the given sample(s).

Principle

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardised time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard.

The B.O.D. test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water. It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution. The test has its widest application in measuring waste loading to treatment plants and in evaluating the efficiency of such treatment systems.

The test consists in taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles are taken for each concentration and three concentrations are used for each sample. One set of bottles is incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content (D_1) in the other set of bottles will be determined immediately. At the end of 5 days, the dissolved oxygen content (D_2) in the incubated set of bottles is determined.

$$\text{Then, mg/L B.O.D.} = \frac{(D_1 - D_2)}{P}$$

where, P = decimal fraction of sample used.

D_1 = dissolved oxygen of diluted sample (mg/L), immediately after preparation.

D_2 = dissolved oxygen of diluted sample (mg/L), at the end of 5 days incubation.

Among the three values of B.O.D. obtained for a sample select that dilution showing the residual dissolved oxygen of at least 1 mg/L and a depletion of at least 2 mg/L. If two or more dilutions are showing the same condition then select the B.O.D. value obtained by that dilution in which the maximum dissolved oxygen depletion is obtained.

Apparatus

1. B.O.D. bottles 300 mL capacity
2. B.O.D. incubator
3. Burette
4. Pipette
5. Air compressor
6. Measuring cylinder etc.

Reagents

1. Distilled water
2. Phosphate buffer solution
3. Magnesium sulphate solution
4. Calcium chloride solution
5. Ferric chloride solution
6. Acid and alkali solution
7. Seeding
8. Sodium sulphite solution
9. Reagents required for the determination of D.O.

Procedure

1. Place the desired volume of distilled water in a 5 litre flask (usually about 3 litres of distilled water will be needed for each sample).
2. Add 1 mL each of phosphate buffer, magnesium sulphate solution, calcium chloride solution and ferric chloride solution for every litre of distilled water.
3. Seed the sample with 1–2 mL of settled domestic sewage.
4. Saturate the dilution water in the flask by aerating with a supply of clean compressed air for at least 30 minutes.
5. Highly alkaline or acidic samples should be neutralised to pH 7.
6. Destroy the chlorine residual in the sample by keeping the sample exposed to air for 1 to 2 hours or by adding a few mL of sodium sulphite solution.
7. Take the sample in the required concentrations. The following concentrations are suggested:

Strong industrial waste	: 0.1, 0.5 and 1 per cent
Raw and settled sewage	: 1.0, 2.5 and 5 per cent
Oxidised effluents	: 5, 12.5 and 25 per cent
Polluted river water	: 25, 50 and 100 per cent

8. Add the required quantity of sample (calculate for 650 mL dilution water the required quantity of sample for a particular concentration) into a 1000 mL measuring cylinder. Add the dilution water up to the 650 mL mark.
9. Mix the contents in the measuring cylinder.
10. Add this solution into two B.O.D. bottles, one for incubation and the other for determination of initial dissolved oxygen in the mixture.

11. Prepare in the same manner for other concentrations and for all the other samples.
12. Lastly fill the dilution water alone into two B.O.D. bottles. Keep one for incubation and the other for determination of initial dissolved oxygen.
13. Place the set of bottles to be incubated in a B.O.D. incubator for 5 days at 20°C. Care should be taken to maintain the water seal over the bottles throughout the period of incubation.
14. Determine the initial dissolved oxygen contents in the other set of bottles and note down the results.
15. Determine the dissolved oxygen content in the incubated bottles at the end of 5 days and note down the results.
16. Calculate the B.O.D. of the given sample.

Note: The procedure for determining the dissolved oxygen content is same as described in the experiment under "Determination of dissolved oxygen".

Observation

Sample no. or description	***Concentration***	***Dissolved oxygen content mg/L***				***B.O.D. mg/L (5 days 20°C)***
		Initial (D_1)		***Final (D_2)***		
		Bottle no.	***D.O. value***	***Bottle no.***	***D.O. value***	

Note: B.O.D. value in mg/L $= \left(\frac{D_1 - D_2}{P}\right)$

If concentration is 0.1 per cent, then $P = \left(\frac{0.1}{100} = 0.001\right)$ and so on.

Sample calculation

D_1 = Initial Dissolved Oxygen = ... mg/L

D_2 = Dissolved Oxygen at the end of 5 days = ... mg/L

P = Decimal fraction of sample used = ...

Therefore, mg/L of B.O.D. $= \frac{D_1 - D_2}{P} =$ .....

Results

Sample no. or description	*mg/L 5 days B.O.D. at 20°C*

Discussion

Questions

1. What use is made of the B.O.D. test in water pollution control?
2. List five requirements, which must be completed with, in order to obtain reliable B.O.D. data.
3. List five requirements of satisfactory dilution water for B.O.D. test.
4. What are the three methods that can be used to control nitrification in the 5 days B.O.D. test at 20°C?
5. What are the factors affecting the rate of biochemical oxidation in the B.O.D. test?

Experiment No. _____ **Date** _____

DETERMINATION OF AVAILABLE CHLORINE IN BLEACHING POWDER

Aim

To determine the available chlorine in the given sample of bleaching powder by the iodometric method.

Principle

Bleaching powder is commonly used as a disinfectant. The chlorine present in the bleaching powder gets reduced with time. So, to find the exact quantity of bleaching powder required, the amount of available chlorine in the sample must be found out.

Chlorine will liberate free iodine from potassium iodide solution when its pH is 8 or less. The iodine liberated, which is equivalent to the amount of active chlorine, is titrated with standard sodium thiosulphate solution using starch as indicator.

Apparatus

1. Mortar and pestle
2. Volumetric flask
3. Burette
4. Pipette
5. Erlenmeyer flask.

Reagents

1. Concentrated glacial acetic acid
2. Standard sodium thiosulphate solution (0.025 N)
3. Potassium iodide
4. Starch indicator
5. Iodine solution (0.025 N).

Procedure

1. Dissolve 1g bleaching powder in 1 litre of distilled water in a volumetric flask, and stopper the container. (This can be done by first making a paste of the bleaching powder with mortar and pestle.)

2. Place 5 mL acetic acid in an Erlenmeyer flask and add about 1g potassium iodide crystals. Pour 25 mL of bleaching powder solution prepared above and mix with a stirring rod.
3. Titrate with 0.025 N sodium thiosulphate solution until a pale yellow colour is obtained. (Deep yellow changes to pale yellow.)
4. Add 1mL of starch solution and titrate until the blue colour disappears.
5. Note down the volume of sodium thiosulphate solution added (V_1).
6. Take a volume of distilled water corresponding to the sample used.
7. Add 5 mL acetic acid, 1g potassium iodide and 1 mL starch solution.
8. If blue colour occurs, titrate with 0.025 N sodium thiosulphate solution until the blue colour disappears.
9. Record the volume of sodium thiosulphate solution added (A_1).
10. If no blue colour occurs, titrate with 0.025 N iodine solution until a blue colour appears. Note down the volume of iodine (A_2).
11. Then, titrate with 0.025 N sodium thiosulphate solution till the blue colour disappears. Record the volume of sodium thiosulphate solution added (A_3). Note down the difference between the volume of iodine solution and sodium thiosulphate as A_4(A_4=A_2 – A_3).

Note: Blank titration is necessary to take care of the oxidising or reducing reagents' impurities.

Observation

Bleaching powder solution × Standard sodium thiosulphate solution (0.025 N)

Trial no.	*Volume of bleaching powder solution (mL)*	*Burette reading*		*Volume of titrant (mL)*
		Initial	*Final*	

Distilled water × Standard sodium thiosulphate solution (0.025 N)

Trial no.	Volume of distilled water (mL)	Burette reading		Volume of titrant (mL)
		Initial	Final	

Distilled water × Standard iodine solution (0.025 N)

Trial no.	Volume of distilled water (mL)	Burette reading		Volume of titrant (mL)
		Initial	Final	

Calculation

$$\text{mg of } Cl_2/\text{mL (B)} = \frac{(V - A_1) \text{ or } (V+A_4)\times N\times 35.46}{\text{mL of bleaching powder solution taken}}$$

1000 mL of bleaching powder solution contains 1000 × B mg of Cl_2

i.e., 1000 mg bleaching powder contains 1000 B mg of Cl_2

therefore, 100 mg of bleaching powder contains = $\frac{1000\times B}{10}$

% of chlorine available = ...

Results

Available chlorine in the given bleaching powder is. ……%

Discussion

Questions

1. What is disinfection? Differentiate between disinfection and sterilisation?
2. Why do we prefer chlorination over other methods of disinfection?
3. Discuss the effect of pH of water and organic matter of water on efficiency of disinfection by chlorine.
4. What is electro-katadyn process?
5. By use of appropriate equilibrium equations show why the addition of chlorine tends to decrease the pH of water, while hypochlorite tends to increase the pH.

Experiment No. _____ **Date** _____

TEST FOR RESIDUAL CHLORINE

A. OTA METHOD

Aim

To determine the amount of free residual chlorine, combined residual chlorine and total residual chlorine present in the given samples of chlorinated water.

Principle

Orthotolidine (OT) is an aromatic compound that is oxidised in acid solution by chlorine, chloramines and other oxidising agents to produce a yellow coloured compound, holoquinone at pH less than 1.8, the intensity of colour being proportional to the amount present. Orthotolidine-arsenite (OTA) method is based upon the fact that free residuals chlorine react instantaneously and chloramines slowly (5 minutes) with orthotolidine. Total residual chlorine is measured with readings after 5 minutes have elapsed. A second test is made in which a reducing agent (sodium arsenite) is added within 5 seconds after 'OT' is added. This allows time for free chlorine to react before the arsenite is added. The arsenite being a much stronger reducing agent than 'OT', reduces the chloramines instantaneously stopping further action with OT. The yellow colour developed in this case is primarily due to the residual chlorine. Then,

Total residual chlorine – Free residual chlorine = Combined residual chlorine

Note: Nitrates and some other oxidising agents also react with 'OT' producing holoquinone. If such materials are present, correction should be applied to account for them.

Apparatus

1. Chloroscope 2. Cuvettes 3. Comparator discs.

Reagents

1. Orthotolidine reagent 2. Arsenite reagent.

Procedure

1. Pour the sample into the middle chamber of the cell of the chloroscope. Add 0.5 mL of 'OT' and mix well with the plunger. Fill the outer chambers with chlorine free distilled water. Wait for 5 minutes. Put the yellow coloured discs in position and compare the colour. Note the reading (A).
2. As in step 1 first add 'OT', mix quickly and immediately add arsenite reagent. Note the reading (B).

Note: If the colour is not in the range of the given discs, the experiment may be done by diluting the sample with distilled water and then multiplying the result by the dilution factor.

Observation

Sample no.	*Trial no.*	*A*	*B*	*C*

Calculation

Total residual chlorine = A mg/L = ...

Free residual chlorine = B mg/L = ...

Combined residual chlorine = (A – B) mg/L = ...

Results

Sample description	*Total residual chlorine mg/L*	*Free residual chlorine mg/L*	*Combined residual chlorine mg/L*

Discussion

B. STARCH IODIDE METHOD

Aim

To determine the amount of total residual chlorine present in the given samples of chlorinated water by starch iodide method.

Principle

Chlorination of water supply is done to destroy or deactivate disease-producing micro-organisms. It will also improve the quality of water by reacting with ammonia, iron, manganese, sulphide and some organic substances. The residual chlorine is maintained in water to promote the primary purpose of chlorination. This method of determination depends upon the oxidising power of free and combined chlorine residuals. Chlorine will liberate free iodine from potassium iodide solution at pH 8 or less. The liberated iodine is titrated against standard sodium thiosulphate solution using starch indicator.

Apparatus

1. Burettes 2. Pipettes 3. Erlenmeyer flask.

Reagents

1. Concentrated glacial acetic acid
2. Potassium iodide
3. Starch indicator
4. Iodine solution 0.025 N
5. Standard sodium thiosulphate solution (0.025 N).

Procedure

1. Take 25 mL of sample in an Erlenmeyer flask.
2. Add 5 mL acetic acid to bring pH in the range 3–4.
3. Add 1g of potassium iodide and mix thoroughly.
4. Titrate with 0.025 N sodium thiosulphate solution until a pale yellow colour is obtained.
5. Add 1mL of starch solution and titrate until the blue colour disappears. Note down the volume of sodium thiosulphate solution added (V_1).
6. Take a volume of distilled water corresponding to the sample used. Add 5 mL acetic acid, 1 g potassium iodide and 1mL starch solution.
7. If blue colour occurs, titrate with 0.025 N sodium thiosulphate solution until the blue colour disappears. Record the volume of sodium thiosulphate solution added (A_1).
8. If no blue colour occurs, titrate with 0.025 N iodine solution until the blue colour appears (A_2).
9. Then titrate with 0.025 N sodium thiosulphate solution till the blue colour disappears. Record the volume of sodium thiosulphate solution added (A_3). Note down the difference between the volume of iodine solution and sodium thiosulphate as A_4.

Note: Blank titration is necessary to take care of the oxidising or reducing reagents' impurities.

Observation

Chlorinated sample × Standard sodium thiosulphate solution (0.025 N)

Sample no.	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of titrant (mL)*
			Initial	*Final*	

Distilled water × Standard sodium thiosulphate solution (0.025 N)

Sample no.	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of titrant (mL)*
			Initial	*Final*	

Distilled water × Standard iodine solution (0.025 N)

Sample no.	*Trial no.*	*Volume of sample (mL)*	*Burette reading*		*Volume of titrant (mL)*
			Initial	*Final*	

Calculation

$$\text{Residual chlorine in mg/L} = \frac{(V - A_1) \text{ or } (V+A_4)\times N\times 35.46\times 1000}{\text{mL of sample}} = ...$$

Results

Sample no. or description	*Total residual chlorine mg/L*

Discussion

Questions

1. What are the different factors that affect the degree of disinfection?
2. Differentiate between free residual chlorine and combined residual chlorine.
3. The dosage of chlorine should be proper. Why?
4. No modification of OTA test is necessary when chloramines are present in water. Why?
5. What are the modifications made in the OTA test, if iron, manganese, etc., are present in water?

Experiment No. _____ **Date** _____

TEST FOR COLIFORMS IN WATER

Aim

To find the Most Probable Number (MPN) of bacterial density by *E.coli* test.

Principle

Coliform group comprises of all the aerobic, facultative and anaerobic gram-negative non-spore forming rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. The standard test for this group may be carried out either by multiple tube fermentation technique or by membrane filter technique. The *E.coli* test by multiple tube fermentation technique consists of 3 phases – presumptive, confirmed and completed.

Escherichia coli (E.coli) for the purpose of sanitary examination of water, is defined as a gram-negative, non-spore forming rod which is capable of fermenting lactose with the production of acid and gas at 35°C in less than 48 hours, which produces indole peptone water containing tryptophan, which is incapable of utilising sodium citrate as its sole source of carbon, which is incapable of producing acetyl methyl carbinol, and which gives a positive methyl red test. The results are expressed in terms of MPN (Most Probable Number), which is based on certain probability formulae. The estimate may give a value greater than the actual number of coliform present. The accuracy of any single test depends on the number of tubes fermented. This method helps in describing the sanitary quality of water.

The safety of the water is generally judged from the knowledge of sanitary condition and mentioned by the number of samples yielding positive or negative results. If more than 95% should yield negative results, the safety is usually assured. The scheme of the MPN test is given as follows:

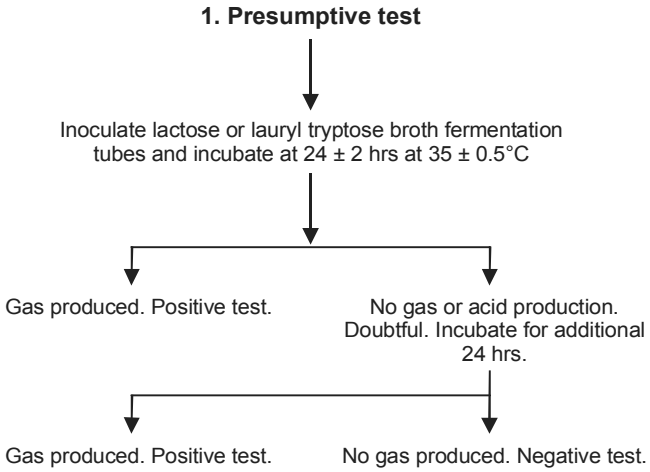
Apparatus

1. Fermentation tubes
2. Petri dishes
3. Autoclave
4. Incubator
5. Test tubes
6. Pipettes
7. Measuring jars
8. Inoculating equipments
9. Media preparation utensils etc.

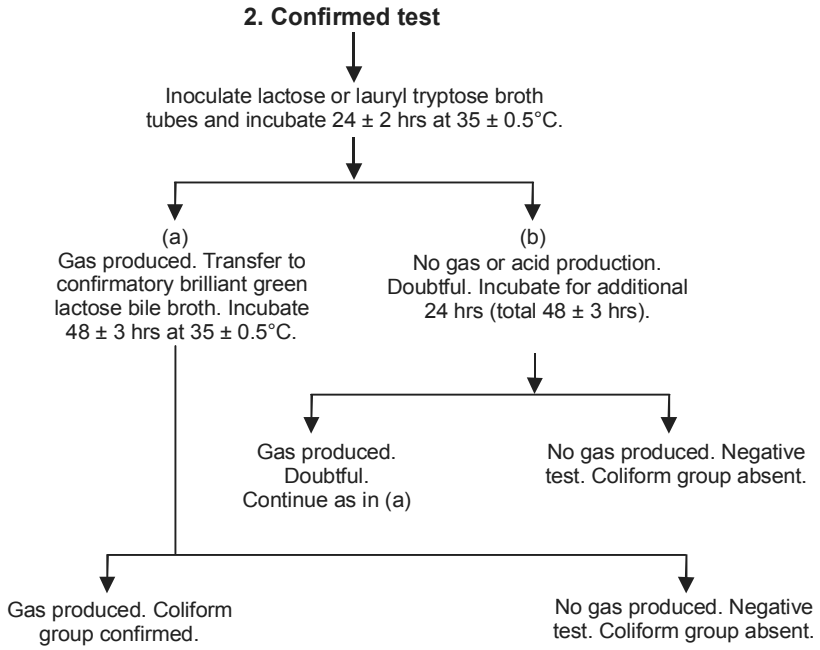
Reagents

1. Lactose broth
2. Lauryl tryptose broth
3. Brilliant green lactose bile broth
4. Endo agar
5. Eosin methylene blue agar etc.

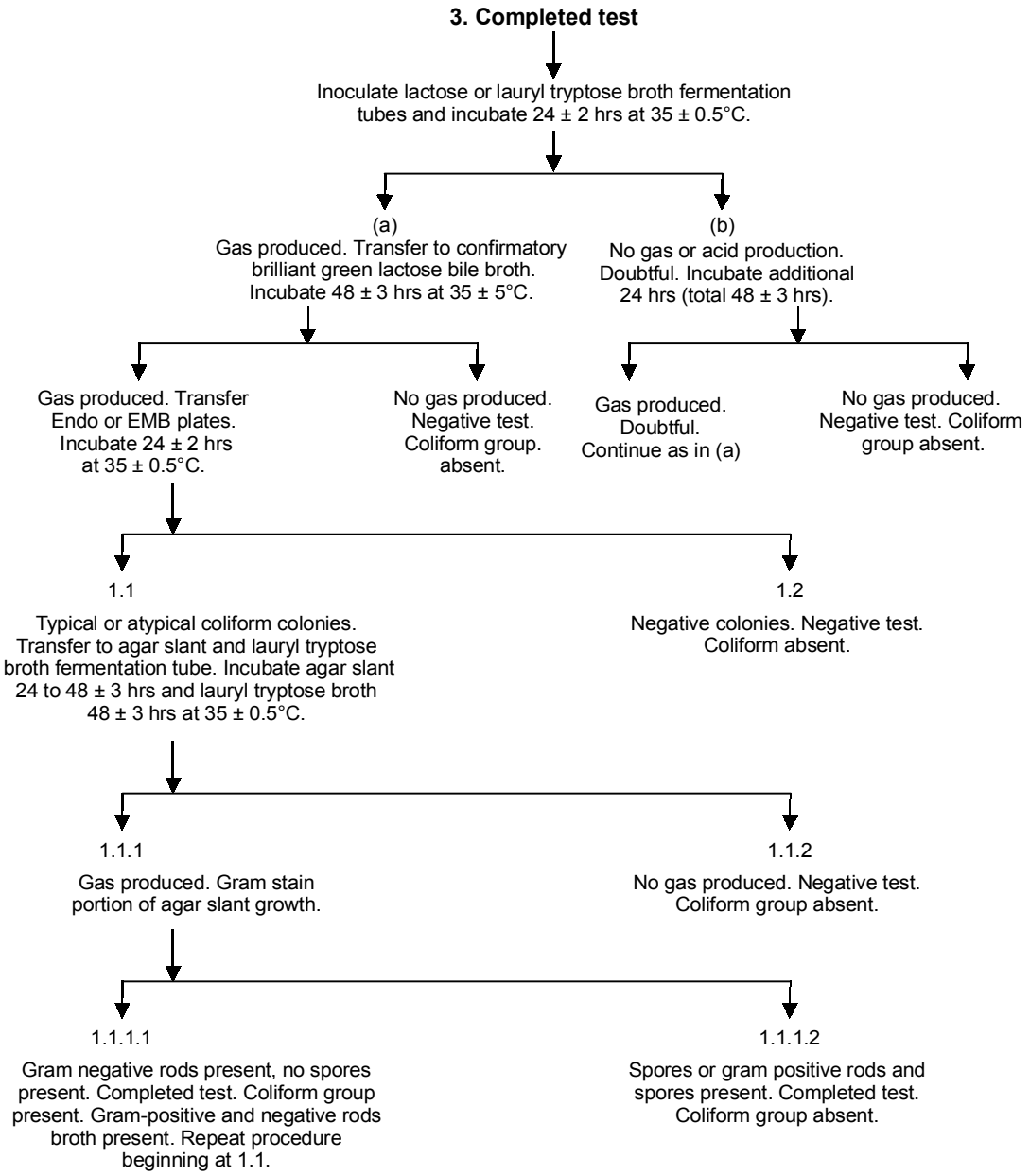
SCHEMATIC OUTLINES FOR THE E.COLI TEST PROCEDURE



Schematic outline of presumptive test for coliform detection.



Schematic outline for presumptive and confirmed test for coliform detection.



Schematic outline of presumptive, confirmed and completed test for total coliform detection.

Procedure

General

Clean and sterilise all the glasswares.

Presumptive Test

1. Inoculate a series of fermentation tubes with appropriate graduated quantities (multiples and sub-multiples of 10) of the water to be tested. The concentration of nutritive ingredients in the mixture of the medium should conform to the specifications. The partitions of the water sample used for inoculating lactose or lauryl tryptose broth fermentation tubes will vary in size and number with the character of the water under examination. Usually, decimal multiples and sub-multiples of 1mL of the sample are selected. Inoculate 10 mL portion of each water sample provided into different one of the three large tubes containing 10 mL of lactose or lauryl tryptose broth which has been prepared with twice the normal concentration of constituent to allow for dilution. Inoculate 1.0 mL and 0.1 mL of water into small tubes (two sets of three each) of single strength lactose or lauryl tryptose broth.
2. Incubate the inoculated fermentation tubes at 35±0.5°C. At the end of 24±2 hrs shake each tube gently and examine and if no gas is formed, repeat this test at the end of 48±3 hrs.
3. Record the presence or absence of gas formation at each examination of the tubes. Formation within 48±3 hrs of gas in any amount in the inverted fermentation tubes constitutes a positive presumptive test. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial in the fermentation tubes. Presumptive test without confirmation should not be used routinely except in the analysis of heavily polluted water, sewage or other waste, which are not suitable for drinking purpose.

Confirmed Test

1. Lactose or lauryl tryptose broth may be used for primary fermentation in presumptive test to avoid false positive results.
2. Brilliant green lactose bile broth fermentation tubes are used in confirmed test.
3. Submit all primary fermentation tubes showing any amount of gas at the end of 24 hrs incubation to the confirmed test.
4. Gently shake primary fermentation tube showing gas formation and with a sterile metal loop, transfer one loop full of medium to a fermentation tube containing brilliant green lactose bile broth.
5. Incubate the inoculated brilliant green lactose bile broth tube for 48±3 hrs at 35±0.5°C.
6. The formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time within 48±3 hrs constitutes a positive confirmed test.
7. If no gas is formed, it is a negative confirmed test and *E.coli* is absent.

Completed Test

Completed test is the next step following the confirmed test. It is applied to the brilliant green lactose bile broth fermentation tubes showing gas in the confirmed test.

1. Streak one or more endo or Eosin Methylene Blue (EMB) agar plates (taken in Petri dishes) from each tube of brilliant green lactose bile broth showing gas.
2. While streaking it is essential to ensure the presence of some discrete colonies separated by at least 0.5 cm from one another.
3. Insert the end of the streaking needle into the liquid in the tube to a depth of 5 mm.

4. Streak the plate by bringing only the curved section of the needle in contact with the agar surface so that the latter will not be scratched or torn.
5. Incubate the Petri dishes (inverted) at 35 ± 0.5°C for 24 ± 2 hrs.
6. The colonies developing on endo or eosin methylene blue agar may be typical (unnucleated, with or without metallic sheen) atypical (opaque, unnucleated, mucoid, pink after incubation for 24 hrs) or negative (all others).
7. From each of these plates fish out one or two colonies and transfer to lauryl tryptose broth fermentation tubes and to nutrient agar slants.
8. Incubate the secondary broth tubes and agar slants at 35 ± 0.5°C for 24 ± 2 hrs or 48 ± 3 hrs and if gas is not produced in 24 hrs gram stained preparation from these agar slant cultures are made.
9. The gas formation in the secondary lauryl tryptose broth tubes and the demonstration of gram-negative non-spore forming rod shaped bacteria in agar culture may be considered a satisfactory positive completed test.
10. If after 48 ± 3 hrs gas is produced in the secondary fermentation tubes and no spore of gram positive rod are found on the slant, the test may be considered as positive completed test and this demonstrates the presence of coliform organisms.

Differentiation of *E. coli* and *A. aerogenes* on eosin or EMB agar can be done by referring the following table.

Differentiation of E.coli and A. aerogenes on eosin or EMB agar

Characteristic	***Escherichia coli***	***Aerobactor aerogenes***
Size	Well isolated colonies are 2–3 mm in diameter.	Well isolated colonies, are larger than those of *E. coli* usually 4–6 mm in diameter.
Confluence	Neighbouring colonies show little	Neighbouring colonies run together tendency to run together. quickly.
Elevation	Colonies are slightly raised, surface is flat or slightly concave, rarely convex.	Colonies are considerably raised and marked by convex, occasionally centre drops precipitedly.
Appearance by transmitted light	Dark, almost black centre that extend across more than three fourths of the diameter of the colony, internal structure of central dark portion is difficult to discern.	Centres are deep brown not as dark as those of *E. coli* and smaller in proportion to the rest of the colony. Striated internal structure is often observed in young colonies.
Appearance by reflected light	Colonies are dark, button like, often concentrically ringed with a greenish metallic sheen.	Much lighter than *E. coli*, metallic sheen is not observed except in a depressed center.

Gram Staining

Reagents

1. Ammonium oxalate-crystal violet (Hucker's)
2. Lugol's solution

3. Counter stain
4. Acetone alcohol.

Procedure

1. Prepare a light emulsion of the bacterial growth on an agar slant in a drop of distilled water on a glass slide.
2. Air-dry or fix by passing the slide through a flame and stain for 1minute with ammonium oxalate-crystal violet solution.
3. Rinse the slide in tap water and then apply Lugol's solution for 1minute.
4. Rinse the stained slide in tap water.
5. Decolorise with acetone alcohol till the stain is just removed.
6. Counter-stain with safranin for 15 seconds and then rinse with tap water.
7. Blot dry with blotting paper and view through the microscope.
8. Cells that decolorise and accept the safranin stain are pink and are defined as gram negative. Cells that do not decolorise but retain the crystal violet stain (deep blue) are defined as gram positive.

Steps in the gram staining is shown in the following table.

Step	*Procedure*	*Results*	
		Gram + ve	*Gram – ve*
Initial stain	Crystal violet for 30 sec.	Stains purple	Stains purple
Mordent	Iodine for 30 sec.	Remains purple	Remains purple
Decolonisation	95% ethanol for 10-20 sec.	Remains purple	Becomes colourless
Counter stain	Safranin for 20-30 sec.	Remains purple	Stains pink

Computation of MPN

The number of positive finding of coliform group organisms resulting from the multiple portion decimal dilution planting should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN). The MPN for the variety of planting series are presented in table in Appendix III. The values are at the 95% confidence limit for each of the MPN determined. These values are prepared for 10,1 and 0.1mL combination. If the combination is 100, 10, 1mL, the MPN is 0.1 times the value in the table. If on the other hand a combination corresponding to 1, 0.1, and 0.01 mL is planted, record MPN as 10 times the value shown in the table.

The MPN for combination not appearing on the table or for other combinations of tubes and dilutions, may be estimated by Thomas' simple formula:

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

Observation

Sample no. or description	*Date and time of observation*	*Date and time of incubation*	*Results after incubation for various volumes of samples inoculated (mL) + ve or – ve*									*Test case*
			10	*10*	*10*	*1*	*1*	*1*	*0.1*	*0.1*	*0.1*	
												Presumptive test 24 hrs
												Presumptive test 48 hrs
												Confirmed test 48 hrs
												Completed test 24 hrs
												Completed test 48 hrs
Number of + ve tubes												
mL of sample in – ve tubes												

Calculation

Case (i)

For three each of 10 mL, 1 mL and 0.1 mL sample concentration combinations

MPN from the MPN table **(Appendix-III)** = ...

Case (ii)

For other combinations and dilutions

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}} = ...$$

Result

MPN/100 mL = ...

Discussion

Questions

1. What are *E.coli*? Are they harmful to human beings? Why is their presence tested in the waters to be supplied for domestic consumption?
2. What is coliform index?
3. Define MPN.

Experiment No. _____ **Date** _____

AMMONIA NITROGEN

Aim

To determine the ammonia nitrogen of the given sample of water.

Principle

Colorimetric method, using Nessler's reagent is sensitive to 20mg/L of ammonia N and may be used up to 5mg/L of ammonia N. Turbidity, colour and substances precipitated by hydroxyl ion interfere with the determination. The sample containing ammonia must be analysed immediately after collection; if not 0.8 M conc. H_2SO_4/L should be added to the sample stored at 4°C.

Direct Nesslerisation

Direct Nesslerisation is used only for purified water, natural water and highly purified effluents, which have low ammonia concentration. In samples that have been properly clarified by a pretreatment method using zinc sulphate and sodium hydroxide, it is possible to obtain a measure of the amount of ammonia N by treatment with Nessler's reagent, which is strongly alkaline solution of potassium mercuric iodide (K_2HgI_4). It combines with NH_3 in alkaline solution to form a yellowish brown colloidal dispersion, whose intensity of colour is directly proportional to the amount of NH_3 present. The yellow colour or reddish brown colour typical of ammonia N can be measured in a spectrophotometer in the wavelength of 400–500 nm with a light path of 1cm.

Apparatus

1. Spectrophotometer, or Nessler tube tall form (50 mL or 100 mL capacity)
2. pH meter

Reagents

1. Zinc sulphate solution
2. EDTA reagent as stabiliser
3. Nessler's reagent
4. Stock ammonium solution 1.00 mL = 1.00 mg

Procedure

1. Residual chlorine is removed by means of a dechlorinating agent (one or two drops sodium thiosulphate solution)
2. 100 mL $ZnSO_4$ solution is added to 100 mL sample and to it is added 0.5 mL of NaOH solution to obtain a pH of 10.5. This is mixed thoroughly.
3. The floc formed is allowed to settle and the clear supernatent is taken for Nesslerisation.
4. If the sample contain Ca or Mg, EDTA reagent is added to 50 mL of sample.
5. To this is added 2 mL of Nessler's reagent (proportional amount to be added (if the sample volume is less).
6. A blank using distilled ammonia free water is treated with Nessler's reagent as above. The absorbance is fixed as zero.
7. Then the sample is put in 1 cm standard tubes of spectrophotometer and the absorbance noted at 400–500 nm wavelengths.
8. A calibration curve is prepared as follows:
   With 0, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 4.0, 5.0 mL of standard NH_4Cl solution in 50 mL distilled water standard diluted samples are prepared.
9. Each sample is Nesslerised as indicated earlier and the absorbance is noted down.
10. A graph with mg of NH_3 along x-axis and absorbance along y-axis is plotted and a straight-line graph is drawn.
11. From the absorbance of a solution of unknown concentration, the μg of NH_3 present can be read from the calibration curve.

Calculation

$$\text{ammonia N in mg/L} = \frac{A}{\text{mL of sample}}$$

where, $A = \mu g$ N found colorimetrically

Observation

The observation is presented in Tables A and B respectively.

Table A: Observation for calibration

Stock ammonia solution in mL	*Ammonia*	*Absorbance*

Table B

Sample no.	*Absorbance*	*Ammonia nitrogen in µg from graph*	*Ammonia nitrogen in mg*

Results

Sample no. or description	*Ammonia nitrogen in mg/L*

Discussion

Questions

1. Discuss the significance of ammonia nitrogen in water.
2. What is the source of ammonia nitrogen in water?

Experiment No. _____ **Date** _____

NITRATE NITROGEN

Aim

To determine the nitrate nitrogen of the given sample of water.

Principle

The reaction with the nitrate and brucine produces yellow colour that can be used for the colorimetric estimation of nitrate. The intensity of colour is measured at 410 nm. The method is recommended only for concentration of 0.1–2.0 mg/L NO_3^-—N. All strong oxidising and reducing agent interfere. Sodium arsenite is used to eliminate interference by residual chlorine; sulphanilic acid eliminates the interferences by NO_2^-—N and chloride interference is masked by addition of excess NaCl. High concentration of organic matter also may interfere in the determination.

Apparatus

1. Spectrophotometer
2. Water bath
3. Reaction tubes
4. Cool water bath

Reagents

1. Stock nitrate solution
2. Standard nitrate solution
3. Sodium arsenite solution
4. Brucine-sulphanilic acid solution
5. Sulphuric acid solution
6. Sodium chloride solution

Procedure

1. Nitrate standards are prepared in the range 0.1–1.0 mg/LN diluting 1.00, 2.00, 4.00, 7.00 and 10.0 mL standard nitrate solution to 10 mL with distilled water.
2. If residual chlorine is present 1 drop of sodium arsenite solution is added for each 0.1 mg Cl_2 and mixed.
3. Set up a series of reaction tubes in test tube stand. Add 10 mL sample or a portion diluted to 10 mL to the reaction tubes.
4. Place the stand in a cool water bath and add 2 mL NaCl solution and mix well.
5. Add 10 mL H_2SO_4 solution and again mix well and allow cooling.

6. The stand is then placed in a cool water bath and add 0.5 ml brucine-sulphanilic acid reagent. Swirl the tubes and mix well and place the tubes in boiling water bath at temperature 95°C.
7. After 20 minutes, remove the samples and immerse in cool water bath.
8. The sample are then poured into the dry tubes of spectrophotometer and read the standards and sample against the reagent blank at 410 nm.
9. Prepare a standard curve for absorbance value of standards (minus the blank) against the concentration of NO_3^-N.
10. Read the concentration of NO_3^-N in the sample from the known value of absorbance.

Calculation

$$\text{Nitrate N in mg/L} = \frac{\mu g\ NO_3^- - N}{mL\ sample}$$

$$NO_3 \text{ in mg/L} = \text{mg/L nitrate N} \times 4.43.$$

Observation

The observation are presented in Tables A and B respectively.

Table A: Observation for calibration

Stock nitrate solution in mL	*Nitrate*	*Absorbance*

Table B

Sample no.	*Absorbance*	*Nitrate nitrogen in μg from graph*	*Nitrate nitrogen in mg*

Results

Sample no. or description	*Nitrate nitrogen in mg/L*

Discussion

Questions

1. In what forms does nitrogen normally occur in natural waters?
2. Discuss the significance of nitrate nitrogen analysis in water pollution control.
3. Differentiate between nitrite nitrogen and nitrate nitrogen.
4. Discuss the application of nitrate nitrogen data.
5. What are the various methods available for the determination of nitrate nitrogen?

Experiment No. _____ **Date** _____

NITRITE NITROGEN

Aim

To determine the nitrite nitrogen of the given sample of water.

Principle

The nitrite concentration is determined through the formation of a reddish-purple azo dye produced at pH 2.0–2.5 by the coupling of diazotised sulphanilic acid with N-(1-naphthyl)-ethylenediamine dihydrochloride.

Apparatus

1. Spectrophotometer

Reagents

1. Sulphanilamide reagent
2. N-(1-naphthyl)-ethylenediamine dihydrochloride solution
3. Hydrochloric acid (1+3)
4. Stock nitrite solution
5. Standard nitrite solution

Procedure

1. To 50 ml clear sample neutralised to pH 7, add 1 ml sulphanilamide solution.
2. Allow the reagent to react for a period of 2–8 minute.
3. Then add 0.1 ml of 1-naphthyl ethylenediamine solutions and mix immediately.
4. Measure the absorbance of the solution after 10 minute at 543 nm at 1 cm light path.
5. Prepare standard calibration curve as in any other case.
6. By noting the absorbance of an unknown sample, the concentration of nitrate can be determined.

Calculation

$$\text{Nitrite N in mg/L} = \frac{\text{mg Nitrite N}}{\text{mL of sample}}$$

Observation

The observation is presented in Tables A and B respectively.

Table A: Observation for calibration

Stock nitrite solution in mL	*Nitrite*	*Absorbance*

Table B

Sample no.	*Absorbance*	*Nitrite nitrogen in µg from graph*	*Nitrite nitrogen in mg*

Results

Sample no. or description	*Nitrite nitrogen in mg/L*

Discussion

Questions

1. Explain why sensitive colorimetric methods are needed for the determination of nitrite nitrogen.
2. Explain the nitrogen cycle.
3. What is the significance of determination of nitrite nitrogen in water?

Experiment No. _____ **Date** _____

KJELDAHL NITROGEN

Aim

To determine the Kjeldahl nitrogen of the given sample of water.

Principle

In the presence of sulphuric acid, potassium sulphate and mercuric sulphate catalyst, the amino nitrogen of many organic materials is converted to ammonium sulphate. After the mercury-ammonium complex, the digestible has been decomposed by sodium thiosulphate, the ammonia is distilled from an alkaline medium and absorbed in boric acid. The ammonia is determined colorimetrically or by titration with a standard mineral acid.

Apparatus

1. Digestion apparatus of 800 mL capacity
2. Distillation apparatus
3. Spectrophotometer

Reagent

1. All reagents listed for the determination of ammonia N
2. Digestion reagent
3. Phenolphthalein indicator
4. Sodium hydroxide-sodium thiosulphate reagent
5. Borate buffer solution
6. Sodium hydroxide 6 N

Procedure

1. Place a measured sample into a digestion flask. Dilute the sample to 300 mL, and neutralise to pH 7. Sample size is determined as follows:

Sample size determination

Organic nitrogen in sample (mg/L)	*Sample size (mL)*
0-1	500
1-10	250
10-20	100
20-50	50
50-100	25

2. Add 25 mL borate buffer and 6 N NaOH until pH 9.5 is reached.
3. Add a few glass beads and boil off 300 mL.
4. Cool and add carefully 50 mL digestion reagent. After mixing heat under a hood until the solution clean to a pale straw colour.
5. Digest for another 30 minute and allow the flask and contents cool.
6. Dilute the contents to 300 mL and add 0.5 mL phenolphthalein solution.
7. Add sufficient hydroxide-thiosulphate reagent to form an alkaline layer at the bottom of the flask.
8. Connect the flashed to the steamed out distillation apparatus and more hydroxide-thiosulphate reagent. If a red phenolphthalein colour fails to appear at this stage.
9. Distilled and collect 200 mL distillate below the surface of boric acid solution. Extend the lip of condenser well bellow the level of boric acid solution.
10. Determine the ammonia as described earlier by taking 50 mL portion of the distillate.
11. Carry out a similar procedure for a blank and apply the necessary correction.

Observation

The observation is presented in Tables A and B respectively.

Table A: Observation for calibration

Stock ammonia solution in mL	*Ammonia*	*Absorbance*

Table B

Sample no.	*Absorbance*	*Ammonia nitrogen in μg from graph*	*Ammonia nitrogen in mg*

Calculation

$$\text{Organic N in mg/L} = \frac{A \times 1000}{\text{mL of sample}} \times \text{B and C}$$

where, A = mg N found colorimetrically

B = mL of total distillate collected including H_3BO_3

C = mL of distillation taken for Nesslerisation.

Results

Sample no. or description	*Organic nitrogen in mg/L*

Discussion

Questions

1. What is the difference between Kjeldahl nitrogen and albuminoidal nitrogen?
2. In which form the organic nitrogen exists in domestic wastewater?

Experiment No. _____ **Date** _____

DETERMINATION OF FLUORIDE IN WATER

Aim

To determine the fluorides present in water.

Principle

Fluorides in excessive quantities and absence of fluorides in water, both create problems. A disfigurement in teeth of humans known as mottled enamel or dental fluorosis is occured in those, who consume waters with fluoride content in excess of 1.0 mg/L. It has been scientifically established that 0.8–1.0 mg/L of fluorides is essential in potable water. Thus, absence or low fluoride content may cause dental caries in the consumers.

Fluorides are measured by colorimetric methods. Fluorides are separated out by distillation, if interfering substances are present. Fluorides are analysed by a method that involves the bleaching of a performed colour by the fluoride ion. The performed colour is the result of the action between zirconium ion and alizarin dye. The colour produced is referred to a lake and the intensity of colour produced is reduced if the amount of zirconium present is decreased. Fluoride ion combines with zirconium ion to form a stable complex ion ZrF_6^{--}, and the intensity of the colour lake decreases accordingly. The reaction is as follows:

$$\underset{\text{(reddish colour)}}{\text{Zr\_alizarin lake}} + 6F \longrightarrow \underset{\text{(yellow)}}{\text{alizarin}} + ZrF_6^{--}$$

The bleaching action is the function of the fluoride ion concentration and is directly proportional to it. Thus, Beer's law is satisfied in and inverse manner.

Apparatus

1. Spectrophotometer or colour comparator

Reagents

1. Standard fluorides solution 1 mL = 10 µgF.
2. Zirconyl-alizarin reagent.

3. Mixed acid solution.
4. Acid-zirconyl-alizarin reagent.
5. Sodium arsenite solution.

Procedure

1. If residual chlorine is present, remove the same by adding one drop of arsenite per 0.1 mg Cl and mix.
2. Prepare a series of standard by diluting various volume of standard fluoride solution (1 ml =10 µgf) to 100 mL in tubes. The range should be such that it is between 0 and 1.4 mg/L.
3. To 50 mL of each standard add 10 mL mixed acid-zirconyl-alizarin reagent.
4. Set the spectrophotometer to a wavelength of 570 nm.
5. Adjust the spectrophotometer to zero absorbance with the reference solution i.e., distilled water with reagent.
6. Plot the concentration along x-axis and absorbance along y-axis and obtain a calibration curve.
7. Take 50 mL of the sample and add 10 mL of mixed acid-zirconyl-alizarin reagent and mix well.
8. Place the solution in the spectrophotometer and read the absorbance.
9. By referring the calibration curve, the concentration for the observed absorbance is read out.
10. Repeat the procedure with dilute samples.

Observation

The observation is presented in Tables A and B respectively.

Table A: Observation for calibration

Stock fluoride solution in mL	*Fluoride*	*Absorbance*

Table B

Sample no.	*Absorbance*	*Fluoride in µg from graph*	*Fluoride in mg*

Calculation

$$F \text{ in mg/L} = \frac{A \times B}{V \times C}$$

where,

A = µgF determined
B = sample dilute to this volume
C = portion taken for colour development
V = mL of sample.

Results

Sample no. or description	*Fluoride in mg/L*

Discussion

Questions

1. Discuss the significance of high fluorides in water supplies.
2. Discuss the significance of low fluorides in water supplies.
3. What is meant by fluoridation of water? How this can be done?
4. Explain an economical defluoridation method for drinking water supplies.
5. What are the various methods for the determination of fluoride in water?
6. Discuss the application of fluoride data.

Experiment No. _____ **Date** _____

DETERMINATION OF ACIDITY OF WATER

Aim

To determine the acidity of the given sample of water.

Principle

Acidity of water is its quantitative capacity to neutralise a strong base to a designated pH. Strong minerals acids, weak acids such as carbonic and acetic and hydrolysing salts such as ferric and aluminium sulphates may contribute to the measured acidity. According to the method of determination, acidity is important because acid contributes to corrosiveness and influences certain chemical and biological processes. It is the measure of the amount of base required to neutralise a given sample to the specific pH.

Hydrogen ions present in a sample as a result of dissociation or hydrolysis of solutes are neutralised by titration with standard alkali. The acidity thus depends upon the end point pH or indicator used. Dissolved CO_2 is usually the major acidity component of unpolluted surface water. In the sample, containing only carbon dioxide-bicarbonate-carbonate, titration to pH 8.3 at 25°C corresponds to stoichiometric neutralisation of carbonic acid to carbonate. Since the colour change of phenolphthalein indicator is close to pH 8.3, this value is accepted as a standard end point for the titration of total acidity. For more complex mixture or buffered solution fixed end point of pH 3.7 and pH 8.3 are used. Thus, for standard determination of acidity of wastewater and natural water, methyl orange acidity (pH 3.7) and phenolphthalein acidity (pH 8.3) are used.

Thus, in determining the acidity of the sample the volumes of standard alkali required to bring about colour change at pH 8.3 and at pH 3.7 are determined.

Apparatus

1. Burette
2. Pipette
3. Erlenmeyer flasks
4. Indicator solutions

Reagents

1. CO_2 free water
2. Standard NaOH solution 0.02 N

3. Methyl orange indicator solution
4. Phenolphthalein indicator solution
5. Sodium thiosulphate 0.1 N.

Procedure

1. 25 mL of sample is pipette into Erlenmeyer flask.
2. If free residual chlorine is present, 0.05 mL (1 drop) of 0.1 N thiosulphate solution is added.
3. 2 drops of methyl orange indicator is added.
4. These contents are titrated against 0.02 N hydroxide solution. The end point is noted when colour change from orange red to yellow.
5. Then two drops of phenolphthalein indicator is added and titration continued till a pink colour just develops. The volumes of the titrant used are noted down.

Observation

0.02 N NaOH × Sample (Methyl orange/phenolphthalein indicator)

Description of sample	*Trial no.*	*Burette reading*		*Volume of NaOH used A*
		Initial	*Final*	

Calculation

$$\text{Acidity in mg/L as } CaCO_3 = \frac{A \times B \times 50{,}000}{V}$$

where, A = mL of NaOH titrant

B = normality of NaOH

V = mL of the sample.

Results

Sample no.	*Acidity in mg/L as $CaCO_3$*

Discussion

Questions

1. Discuss the source and nature of acidity.
2. Discuss the significance of carbon dioxide and mineral acidity.
3. Can the pH of a water sample be calculated from a knowledge of its acidity? Why?
4. Can the carbon dioxide content of a wastewater sample known to contain significant concentrations of acetic acid be determined by the titration procedure? Why?

Experiment No. _____ **Date** _____

DETERMINATION OF C.O.D. IN WATER

Aim

To determine the Chemical Oxygen Demand (C.O.D.) for given sample.

Principle

Potassium dichromate is a powerful oxidising agent in acidic medium and is obtained in high state of purity.

The reaction involved is:

$$C_nH_aO_b + cCr_2O_7^{2-} + 8cH^+ = nCO_2 + \frac{a + 8c}{2} H_2O + 2cCr^{3+}$$

where, $c = 2/3n + a/6 - b/3$

C.O.D. results are reported in terms of mg of oxygen. N/8 or 0.125 N solution of oxidising agent is used in the determination. Normality double the strength is used. This allows the use of larger samples. Thus, each ml of 0.25 N solution dichromate is equivalent to 2 mg of oxygen. An excess of oxidising agent is added, the excess is determined by another reducing agent such as ferrous ammonium sulphate. An indicator ferroin is used in titrating the excess dichromate against ferrous ammonium sulphate. Blanks are used also treated and titrated to get the correct value of C.O.D.

Apparatus

1. Reflux apparatus 2. Burettes 3. Pipettes

Reagents

1. Standard potassium dichromate solution 0.25 N.
2. Sulphuric acid reagent.
3. Standard ferrous ammonium sulphate.
4. Ferroin indicator solution.
5. Mercuric sulphate.
6. Sulphuric acid crystals.

Procedure

1. Place 50.0 mL of sample in a 500 mL refluxing flask.
2. Add 1g mercuric sulphate and a few glass beads.
3. Add sulphuric acid to dissolve the mercuric sulphate and cool.
4. Add 25.0 ml 0.25 N potassium dichromate solution and mix well.
5. Attach the flask to the condenser and start the cooling water.
6. Add the remaining acid reagent (70 mL) through the open end of condenser and mix well.
7. Apply heat and reflux for 5 hours.
8. Cool and wash down the condenser with distilled water.
9. Dilute the mixture to about twice its volume and cool to room temperature.
10. Titrate the excess dichromate with standard ferrous ammonium sulphate using ferroin indicator (2 to 3 drops).
11. The colour change from blue green to reddish indicates the end point.
12. Reflux in the same manner a blank consisting of distilled water of equal volume as that of the sample.

Observation

	Burette reading		***Volume of ferrous ammonium sulphate***
	Initial	***Final***	
Sample			
Blank			

Calculation

$$\text{mg/L C.O.D.} = \frac{(V_1 - V_2)\, N \times 8000}{V},$$

where,

V_1 = mL ferrous ammonium sulphate used for blank

V_2 = mL ferrous ammonium sulphate used for sample

N = normality of ferrous ammonium sulphate

V = volume of sample used.

Results

Sample no.	*C.O.D. in mg/L*

Discussion

Questions

1. Differentiate between B.O.D. and C.O.D.
2. Discuss the application of C.O.D. analysis in environmental engineering practice.
3. What are the interferences during C.O.D. test? How this can be eliminated?
4. Why ferroin is used as indicator in the C.O.D. test?
5. Why 0.25 N standard dichromate solution is used in the test?

Experiment No. _____ **Date** _____

DETERMINATION OF OIL AND GREASE

Aim

To determine the quantity of oil and grease present in the given sample of water by partition gravimetric method.

Principle

The oil and grease contents of domestic and certain industrial wastes and the sludge, is of an important consideration in the handling and treatment of these material for ultimate disposal. Knowledge of the quality of the oil and grease present is helpful in proper design and operation of wastewater treatment system. The term grease applies to wide variety of organic substance that is extracted from aqueous solution or suspension by hexane. Hydrocarbons, esters, oils, fats, waxes and high molecular weight fatty acids are the major materials dissolved by hexane. All these material have a greasy feel and are associated with the problems in wastewater treatment related to grease.

Three methods by which oil and grease are estimated are (i) the partition-gravimetric method (ii) partition infrared method, and (iii) the solvent extraction method. Though method (i) does not provide needed precision, it is widely used for routine analysis of samples with high oil and grease contents because of its simplicity and no need of special instrumentation. In method (ii) adequate instrumentation allows for the measurements of as little as 0.2 mg oil and grease. Method (iii) is identical to gravimetric method but it is designed for the samples that might contain volatile hydrocarbons that otherwise would be lost in the solvent removal operation of gravimetric method.

In the Partition-Gravimetric method, dissolved or emulsified oil and grease is extracted from water by intimate contact with trichlorotrifluoroethane; petroleum ether (40/60) or hexane**.**

Apparatus

Beaker, separating funnel, distilling flask, desiccators, vacuum pump.

Reagents

HCl, trichlorotrifluosoethane (freon).

Procedure

1. Collect about 1 litre of sample and mark sample level in bottle for latter determination of sample volume. Acidity to pH 2 or lower; generally, 5 ml HCl is sufficient. Transfer to a separating funnel. Carefully rinse sample bottle with 30 ml trichlorotrifluoroethane and solvent washing to separating funnel.
2. Preferably shake vigorously for 2 minute. However, if it is suspected for stable emulsion shakes gently 5 to 10 minute.
3. Let layer separate out, drain solvent layer through a funnel containing solvent– moistened filter paper into a clean, evacuated distilling flask. If a clear solvent layer cannot be obtained, add 1g Na_2SO_4, if necessary.
4. Extract twice more with 30 ml solvent each time but first rinse sample container with solvent. Combine extracts in evacuated distilling flask and mash filter paper with an additional 10 ml to 20 ml solvent.
5. Distill solvent from distilling flask in a water bath at 70°C. Place flask on water bath at 70°C for 15 minute and draw air through it with an applied vacuum for final 1 minute after the solvent has evaporated. If the residue contains visible water, add 2 ml acetone evaporates on a water bath and repeat the addition and evaporation until all visible water has been removed. Cool in a desiccators for 30 minute and weigh it.

Precautions

No known solvent will dissolve effectively only oil and grease. Solvent removal results in the loss of short chain hydrocarbon and simple aromatic compound by volatilisation, and heavier residual of some effluents may contain significant portion of material that are not extracted with the solvent.

Observation

Temperature of measurement =…....°C

Mass of evacuated flask =……...g

Mass of evacuated flask + residue =……g

Mass of residue =……mg

Volume of sample =……...…mL

Calculation

The amount of oil and grease in the sample can be calculated as,

Oil and Grease (mg/L) = (A – B) 1000/volume of the sample

where, A = mass of evacuated flask and residue (g)

B = mass of evacuated flask (g)

Result

The amount of oil and grease in given water sample = …….. mg/L.

Discussion

Questions

1. Why is it important to remove oil and grease from water?
2. What are the methods available to remove oil and grease from water?

Experiment No. _____ **Date** _____

DETERMINATION OF ODOUR

Aim

To determine the odour of the given sample of water using human olfactory system.

Principle

The extent of odour present in a particular sample of water is measured by a term called odour intensity, which is related with the threshold odour. The threshold odour number represents the dilution ratio at which odour is hardly detectable.

Apparatus

Measuring jar, pipettes etc.

Procedure

1. Take a known volume (A) of odorous water in a measuring jar.
2. Gradually add odour free distilled water to the sample. Addition of water is continued till the sample just lost its odour. Note down the total volume.

Observation and calculation

Sample no.	*Trial no.*	*Volume of odourous water (A mL)*	*Diluted volume (B mL)*	*Threshold odour number (B/A)*

Results

Sample no.	*Threshold odour number*

Discussion

Questions

1. How odour can be controlled?
2. Which instrument is used for odour determination?

Experiment No. _____ **Date** _____

DETERMINATION OF COLOUR

Aim

To determine the colour of given sample of water by direct observation.

Principle

The standard unit of colour is the colour produced by dissolving 1mg of platinum cobalt in 1 litre distilled water. Different standard colour intensities of solutions are prepared by dissolving known amounts of platinum cobalt with distilled water and the colour of sample is compared with the standard colour solutions.

Apparatus

Nessler tubes, volumetric flask, pipettes etc.

Procedure

1. Take 0.1mg of platinum cobalt and dissolve in 100 mL distilled water.
2. Take 1 mL, 2 mL, 3 mL etc. of this solution and dilute to 50 mL, such that we get solutions of concentrations 1/50, 2/50, 3/50, (n/50) mg/L etc. These are the comparator solutions.
3. Take the sample and the comparator solutions in the Nessler tubes and compare the colour.
4. Note down the dilution of the comparator solution having the same colour as the sample.
5. If corresponding colours are not developed, the sample may be diluted to get the corresponding colour.

Observation and calculation

Sample no.	*Dilution factor of comparator solution (n/50)*	*Colour unit (mg/L) (= 50/n)*

Results

Sample no.	*Colour unit in (mg/L)*

Discussion

Questions

1. Define the standard unit of colour.
2. Briefly explain the causes of colour in water.
3. Differentiate between “apparent” and “true” colour.
4. What is the reason for keeping drinking water standard for colour?

APPENDICES

APPENDIX–I. PREPARATION OF REAGENTS AND MEDIA

Reagents for various determinations are prepared as follows:

Alkalinity

1. **0.02 N standard sulphuric acid:** Prepare stock solution approximately 0.1 N by diluting 2.5 mL concentrated sulphuric acid to 1 litre. Dilute 200 mL of the 0.1 N stock solution to 1 litre CO_2 free distilled water. Standardise the 0.02 N acid against a 0.02 N sodium carbonate solution which has been prepared by dissolving 1.06 g anhydrous Na_2CO_3 and diluting to the mark of a 1 litre volumetric flask.
2. **Methyl orange indicator:** Dissolve 500 mg methyl orange powder in distilled water and dilute it to 1 litre. Keep the solution in dark or in an amber coloured bottle.
3. **Phenolphthalein indicator:** Dissolve 5 g phenolphthalein in 500mL ethyl alcohol and add 500 mL distilled water. Then add 0.02 N sodium hydroxide drop-wise until a faint-pink colour appears.
4. **Sodium thiosulphate 0.1 N:** Dissolve 25 g $Na_2S_2O_3.5H_2O$ and dilute to 1 litre.

Hardness

5. **Ammonia buffer solution:** Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 mL concentrated ammonium hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA and dilute to 250 mL with distilled water. Do not store more than a month's supply. Discard the buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ± 0.1 at the end point of titration. Keep the solution in a plastic or resistant glass container.
6. **Eriochrome black T indicator:** Mix 0.5 g Eriochrome black T dye with 4.5 g hydroxylamine hydrochloride. Dissolve this mixture in 100 ml of 95% ethyl or isopropyl alcohol.
7. **Standard EDTA titrant 0.01 M:** Weigh 3.723 g analytical reagent grade EDTA disodium salt ($Na_2H_2C_{10}H_{12}O_8N_2$) and dissolve in distilled water and dilute to 1litre.

Chloride

8. **Potassium chromate indicator:** Dissolve 50 g potassium chromate ($K_2Cr_2O_4$) in a little distilled water. Add silver nitrate solution until a definite red precipitate is formed. Let stand for 12 hours, filter and dilute the filtrate to 1 litre with distilled water.

9. **Standard silver nitrate solution 0.0141 N:** Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1 litre. Standardise against 0.0141 N NaCl. Store in a brown bottle; 1 mL = 500 µg Cl_2.
10. **Standard sodium chloride 0.0141N:** Dissolve 824.1 mg NaCl (dried at 140°C) in chloride free water and dilute to 1 litre. 1mL = 500 µg Cl_2 .
11. **Aluminium hydroxide suspension:** Dissolve 125 g aluminium potassium sulphate in 1 litre water. Warm to 60°C and add 55 mL concentrated NH_4OH slowly with stirring. Let stand for 1 hour, transfer the mixture to a large bottle. When freshly prepared the suspension occupies a volume of approximately 1 litre.

Iron

12. **Hydrochloric acid:** Concentrated HCl.
13. **Hydroxylamine solution:** Dissolve 10 g hydroxylamine hydrochloride salt ($NH_2OH.HCl$) in 100 mL distilled water.
14. **Ammonium acetate buffer solution:** Dissolve 250 g ammonium acetate ($NH_4C_2H_3O_2$) in 150 mL distilled water. Add 700 mL concentrated (glacial) acetic acid.
15. **Sodium acetate solution:** Dissolve 200 g sodium acetate ($NaC_2H_3O_2.3H_2O$) in 800 mL distilled water.
16. **Phenanthroline solution:** Dissolve 100 mg 1, 10-phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) in 100 mL distilled water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops of concentrated HCl are added to the distilled water. 1 mL of this reagent is sufficient for no more than 100 µg Fe.
17. **Stock iron solution:** Add slowly 20 mL concentrated H_2SO_2 to 50 mL distilled water and dissolve 1.404 g ferrous ammonium sulphate [$Fe(NH_4)_2(SO_4)_2.6H_2O$]. Add 0.1 N $KMnO_4$ drop wise until a faint-pink colour persists. Dilute to 1litre with iron free distilled water. Each 1 mL of this solution contains 200 µg Fe.
18. **Standard iron solution:** Pipette 50 mL stock solution into 1 litre volumetric flask and dilute to the mark with distilled water. 1 mL = 10 µg Fe.

Manganese

19. **Special reagent:** Dissolve 75 g mercuric sulphate ($HgSO_4$) in 400 mL concentrated nitric acid (HNO_3) and 200 mL distilled water. Add 200 mL 85% phosphoric acid and 35 mg silver nitrate to the above solution. Dilute the cooled solution to 1 litre.
20. **Ammonium persulphate:** $(NH_4)_2S_2O_3$ solid.
21. **Standard manganese solution:** Prepare a 0.1 N potassium permanganate ($KMnO_4$) solution by dissolving 3.2 g of $KMnO_4$ in distilled water and making it up to 1 litre. Age for several days in sunlight or heat for several hours near the boiling point and then filter through fritted glass filter crucible and standardise against sodium oxalate. Calculate the volume of this solution necessary to prepare 1 litre solution of such strength that 1mL = 50 µg Mn as follows:

$$\text{mL } KMnO_4 = \frac{4.55}{\text{Normality of } KMnO_4}$$

To this solution add 2 to 3 mL concentrated H_2SO_4 and sodium bisulphite solution (10 g $NaHSO_3$ + 100 mL distilled water). Boil to remove excess SO_2, cool and dilute to 1000 mL with distilled water.

Sulphate

22. **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.
23. **Barium chloride:** Barium chloride crystals.
24. **Standard sulphate solution:** Prepare a standard sulphate solution such that 1 mL = 100 µg SO_4. Dissolve 147.9 mg anhydrous Na_2SO_4 in 500 mL distilled water and dilute to 1 litre 1 mL = 100 µg SO_4.

Sulphide

25. **Hydrochloric acid:** Prepare a 6 N solution.
26. **Standard iodine solution 0.025 N:** Dissolve 20–25 g potassium iodide in a little water and add 3.2 g iodine. After the iodine has dissolved, dilute to 1 litre and standardise against 0.025 N sodium thiosulphate using starch as indicator.
27. **Standard sodium thiosulphate 0.025 N:** Dissolve 6.205 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in freshly boiled and cooled distilled water and dilute to 1litre. Preserve by adding 5mL chloroform or 0.4 g NaOH/L or 4 g borax and 5–10 mg HgI_2/L. Standardise this with 0.025 N potassium dichromate solution which is prepared by dissolving 1.226 g potassium dichromate in distilled water and diluted to 1 litre.
28. **Starch indicator:** Add cold water suspension of 5 g soluble starch to approximately 800 mL boiling water with stirring. Dilute to 1 litre, allow to boil for a few minutes and let settle overnight. Use supernatant liquor. Preserve with 1.25 g salicylic acid/litre or by the addition of a few drops of toluene.

Dissolved oxygen

29. **Manganous sulphate solution:** Dissolve 480 g $MnSO_4.4H_2O$, 400 g $MnSO_2.2H_2O$ or 364 g $MnSO_4.H_2O$ in distilled water, filter and dilute to 1 litre.
30. **Alkali-iodide-azide reagent:** Dissolve 500 g NaOH or 700 g KOH and 135 g NaI or 150 g KI in distilled water and dilute to 1 litre. Add 10 g sodium azide (NaN_3) dissolved in 40 mL distilled water. The reagent should not give colour with starch when diluted and acidified.
31. **Sulphuric acid concentrated:** 1mL is equivalent to about 3 mL alkali-iodide-azide reagent.
32. **Standard sodium thiosulphate 0.025 N:** Dissolve 6.205 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in freshly boiled and cooled distilled water and dilute to 1 litre. Preserve by adding 5 mL chloroform or 0.4 g NaOH/L or 4 g borax and 5–10 mg HgI_2/L. Standardise this with 0.025 N potassium dichromate solution which is prepared by dissolving 1.226 g potassium dichromate in distilled water and diluted to 1 litre.
33. **Standard potassium dichromate solution 0.025 N:** A solution of potassium dichromate equivalent to 0.025 N sodium thiosulphate contains 1.226 g/L $K_2Cr_2O_7$. Dry $K_2Cr_2O_7$ at 103°C for 2 hrs before making the solution.
34. **Standardisation of 0.025 N sodium thiosulphate solution:** Dissolve approximately 2 g KI in an Erlenmeyer flask with 100 to 150 mL distilled water. Add 10 mL of H_2SO_4, followed by exactly 20 mL, 0.1 N potassium dichromate solution. Place in the dark for 5 minutes, dilute to approximately 400 mL and titrate with 0.025 N sodium thiosulphate solution, adding starch towards the end of titration. Exactly 20 ml 0.025 N thiosulphate will be consumed at the end of the titration. Otherwise, the thiosulphate solution should be suitably corrected.

35. **Starch Indicator:** Add cold water suspension of 5 g soluble starch to approximately 800 mL boiling water with stirring. Dilute to 1 litre, allow to boil for a few minutes and let settle overnight. Use supernatant liquor. Preserve with 1.25 g salicylic acid/1 litre or by the addition of a few drops of toluene.

BOD

36. **Phosphate buffer solution:** Dissolve 8.5 g potassium dihydrogen phosphate (KH_2PO_4), 21.75 g dipotassium hydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$) and 1.7 g NH_4Cl in about 500 ml distilled water and dilute to 1 litre. The pH of this buffer should be 7.2 without further adjustment. Discard the reagent if there is any sign of biological growth in the stock bottle.
37. **Magnesium sulphate solution:** Dissolve 22.5 g $MgSO_4.7H_2O$ in distilled water and dilute to 1 litre.
38. **Calcium chloride solution:** Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 litre.
39. **Ferric chloride solution:** Dissolve 0.25 g $FeCl_3.6H_2O$ in distilled water and dilute to 1 litre.
40. **Sodium sulphate solution 0.025 N:** Dissolve 1.575 g anhydrous Na_2SO_3 in 1 litre distilled water. This is to be prepared daily.
41. **Seeding:** The standard seed material is settled domestic wastewater that has been stored at 20°C for 24 to 36 hours. A seed concentration of 1–2 mL/L is usually adopted.

Residual chlorine (OTA)

42. **Dissolve 1.35 g orthotolidine dihydrochloride in 500 mL distilled water:** Add this solution with constant stirring to a mixture of 350 mL distilled water and 150 mL concentrated hydrochloric acid. Store the solution in brown bottle.

    Always use an automatic, dropping or safety pipette to measure the necessary volume. Avoid inhalation or exposure to the skin.

Coliform test

43. **Lactose broth:** Beef extract 3 g, peptone 5 g, lactose 5 g and reagent grade distilled water 1 litre. Add these ingredients to reagent grade distilled water, mix thoroughly and heat to dissolve. pH should be 6.8–7.0 after sterilisation.
44. **Lauryl tryptose broth:** Tryptose 20 g, lactose 5 g, K_2HPO_4 2.75 g, KH_2PO_4 2.75 g, NaCl 5 g, sodium lauryl sulphate 0.1 g, reagent grade distilled water 1 litre, sterilise and use. Add dehydrated ingredients to water, mix thoroughly and heat to dissolve. pH should be 6.8 ± 2 after sterilisation.
45. **Endo agar:** Peptone 10 g, lactose 10 g, K_2HPO_4 3.5 g, agar 15 g, sodium sulphite 2.5 g, basic fuchsin 0.5 g, distilled water 1 litre, pH 7.4 after sterilisation.
46. **EMB agar:** Peptone 10 g, lactose 10 g, K_2HPO_4 2 g, agar 15 g, eosin 0.4 g, methylene blue 0.065 g, distilled water 1 litre, pH should be 7.1 after sterilisation.
47. **Brilliant green lactose bile broth:** Peptone 10 g, lactose 10 g, oxgall 20 g, brilliant green 0.0133 g, distilled water 1 litre, pH should be 7.2 after sterilisation and is then ready for use. Store away from direct sunlight to extend the reagent stability to 6 months.

Acidity

48. **NaOH solution 0.02 N:** Dissolve 4 g NaOH in 1 litre water. This gives 0.1 N NaOH solution. Take 200 ml of this 0.1 N solution and make it up to 1 litre to obtain 0.02 N NaOH solution.

49. **Methyl orange indicator:** Dissolve 500 mg methyl orange powder in distilled water and dilute it to 1 litre.
50. **Phenolphthalein indicator:** Dissolve 5 g phenolphthalein disodium salt in distilled water and dilute to 1 litre.
51. **Sodium thiosulphate 0.1 N:** Dissolve 25 g $Na_2S_2O_3.5H_2O$ and dilute to 1 litre distilled water.

COD

52. **Standard potassium dichromate solution 0.25 N:** Dissolve 12.259 g $K_2Cr_2O_7$ primary standard grade previously dried at 103°C for 2 hours and dilute to 1 litre.
53. **Sulphuric acid reagent:** Concentrated H_2SO_4 containing 22 g silver sulphate per 4 kg bottle. Dissolve 22 g Ag_2SO_2 in 4 kg bottle and keep it for 2 days. This is the reagent.
54. **Standard ferrous ammonium sulphate 0.1 N:** Dissolve 39 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 mL conc. H_2SO_4 and cool and dilute to 1 litre. Standardise this against the standard dichromate solution. Dilute 10 mL standard $K_2Cr_2O_7$ solution to about 100 mL. Add 30 mL conc. H_2SO_4 and cool. Titrate with ferrous ammonium sulphate titrant using 2–3 drops of ferroin indicator.

$$\text{Normality} = \frac{\text{mL } K_2Cr_2O_7 \times 0.25}{\text{mL Fe } (NH_4)_2(SO_4)_2}$$

Ammonia N

55. **Zinc sulphate solution:** Dissolve 100 g $ZnSO_4.7H_2O$ and dilute to 1 litre.
56. **EDTA reagent (stabiliser):** Dissolve 50 g EDTA disodium salt in 60 mL of water containing 10 g NaOH.
57. **Nessler's reagent:** Dissolve 100 g HgI_2 and 70 g KI in a small quantity of water and add this mixture slowly with stirring to a cool solution of 160 g NaOH in 500 mL water. Dilute to 1 litre and store in rubber stoppered pyrex glass out of sunlight.
58. **Stock ammonia solution:** Dissolve 3.811 g anhydrous NH_4Cl dried at 100°C in water and dilute to 1 litre. 1 mL = 1.00 mg N and 1.22 mg NH_3.

Nitrate N

59. **Stock nitrate solution:** Dissolve 721.8 mg anhydrous potassium nitrate and dilute to 1 litre with distilled water. 1 mL = 0.1 mg N.
60. **Standard nitrate solution:** Dilute 10 mL stock nitrate solution to 1 litre. 1 mL = 1 μg N
61. **Sodium arsenite solution:** Dissolve 5.0 g $NaAsO_2$ and dilute to 1 litre.
62. **Brucine-sulphanilic acid solution:** Dissolve 1 g brucine sulphate and 0.1 g sulphanilic acid in about 70 mL of hot distilled water. Add 3 mL conc. HCl, cool and make up to 100 mL. This is stable for several months.
63. **Sulphuric acid solution:** Carefully add 500 mL conc. H_2SO_4 to 125 mL distilled water and cool to room temperature.
64. **Sodium chloride solution:** Dissolve 300 g NaCl and dilute to 1litre with distilled water.

Nitrite N

65. **Sulphanilamide reagent:** Dissolve 5 g sulphanilamide in a mixture of 50 mL conc. HCl and about 300 mL distilled water. Dilute to 500 mL with distilled water.
66. **N-(1-naphthyl)-ethylenediamine dihydrochloride solution:** Dissolve 500 mg dihydrochloride in 500 mL distilled water. Store in a dark bottle.
67. **Hydrochloric acid: HCl (1+3)**
68. **Stock nitrite solution:** Dissolve 1.232 g $NaNO_2$ in nitrite free water and dilute to 1 litre. Fresh nitrite from bottle should be taken 1 mL = 250 mg N in the solution. Preserve with 1 mL chloroform.
69. **Standard nitrite solution:** Standardise stock solution. Pipette 50 ml standard 0.05 N $KMnO_4$, 5 mL conc.H_2SO_4 and 50 mL stock nitrite solution in a glass stoppered flask. Discharge the permanganate colour by ferrous ammonium sulphate solution of 0.05 N (19.607 g ferrous ammonium sulphate and 20 mL conc.H_2SO_4 in 1 litre) strength. Carry nitrite free blank through the entire procedure and make necessary corrections. Calculate the nitrite N content of stock solution by the following equation:

$$A = [(B \times C) - (D \times E)] \times 7/F$$

where, A = mg/mL nitrite N in stock solution,
B = total mL standard $KMnO_4$ used,
C = normality of $KMnO_4$ solution,
D = total mL of standard $Fe(NH_4)_2(SO_4)_2$ used,
E = normality of standard $Fe(NH_4)_2(SO_4)_2$,
F = mL of stock $NaNO_2$ solution taken for titration.

Each 1 mL of 0.05 N $KMnO_4$ consumed by the nitrite corresponds to 1.729 μg $NaNO_2$ or 350 μg N.

Organic Nitrogen (to find Kjeldahl Nitrogen)

70. **Digestion reagent:** Dissolve 134 g K_2SO_4 in 650 mL ammonia free distilled water and 200 mL conc.H_2SO_4. Add with stirring a solution prepared by dissolving 2 g red mercuric oxide (HgO) in 25 mL 6N H_2SO_4. Dilute the combined solution to 1 litre.
71. **Sodium hydroxide-sodium thiosulphate reagent:** Dissolve 500 g NaOH and 2 g $Na_2S_2O_3.5H_2O$ in ammonia free distilled water and dilute to 1 litre.
72. **Borate buffer solution:** Add 88 mL 0.1N NaOH solution to 500 mL 0.025 M sodium tetraborate ($Na_2B_4O_7$) solution (5 g $Na_2B_4O_7$ in 1 litre) and dilute to 1 litre.
73. **Sodium hydroxide 6 N:** Dissolve 240 g NaOH in 1 litre ammonia free distilled water.
74. **Standard iodine 0.1 N:** Dissolve 40 g KI in 25 ml distilled water, add 13 g resublimed iodine and stir until dissolved. Transfer to 1 litre volumetric flask and dilute to the mark.

Fluoride

75. **Standard fluoride solution:** Dissolve 221 mg anhydrous sodium fluoride in distilled water and dilute to 1 litre. 1 mL = 100 μg F. This is the stock solution. Pipette 100 mL stock solution and make it up to 1 litre with distilled water to obtain standard solution 1ml = 10 μg F.
76. **Zirconyl-alizarin reagent:** Dissolve 300 mg zirconyl chloride octahydrate ($ZrOCl_2.8H_2O$) in 50 mL distilled water contained in 1 litre glass stoppered volumetric flask. Dissolve 70 mg of 3-alizarin sulphonic acid sodium salt (also called alizarin red S) in 50 mL distilled water and pour slowly into the zirconyl solution while stirring. The resulting solution clears on standing for a few minutes.
77. **Mixed acid solution:** Dilute 101 mL conc. HCl to approximately 400 mL with distilled water. Add carefully 33.3 mL conc. H_2SO_4 to approximately 400 mL distilled water. After cooling, mix the two acids.
78. **Acid-zirconyl-alizarin reagent:** To the clear zirconyl-alizarin reagent in 1 litre volumetric flask, add the mixed acid solution and distilled water to the mark and mix. The reagent changes in colour from red to yellow within an hour.

APPENDIX–II. STANDARDS FOR DRINKING WATER

Requirements	*Acceptable*	*Cause for rejection**
Physical		
1. Turbidity (Turbidity Units)	2.5	10
2. Colour units on platinum cobalt scale	5	25
3. Taste and odour	Not objectionable	
Chemical		
1. pH	7 to 8.5	Less than 6.5 or greater than 9.2
2. Total solids mg/L	500	1500
3. Total Hardness (as $CaCO_3$) mg/L	200	600
4. Calcium (as Ca) mg/L	75	200
5. Magnesium (as Mg) mg/L	30	150
6. Iron (as Fe) mg/L	0.1	1
7. Manganese (as Mn) mg/L	0.05	0.5
8. Copper (as Cu) mg/L	0.05	1.5
9. Zinc (as Zn) mg/L	5.0	15
10. Chlorides (as Cl) mg/L	200	1000
11. Sulphates (as SO_4) mg/L	200	400
12. Phenolic substances (as Phenol) mg/L	0.001	0.002
13. Fluorides (as F) mg/L	1.0	2.0
14. Nitrates (as NO_3) mg/L	45	45
Toxic substances		
1. Arsenic (as As) mg/L	0.05	0.05
2. Chromium (as hexavalent) mg/L	0.05	0.05
3. Cyanides (as CN) mg/L	0.05	0.05
4. Lead (as Pb) mg/L	0.1	0.1
5. Selenium (as Se) mg/L	0.01	0.01
Ratio activity		
1. Alpha emitters, μc/mL	10^{-9}	10^{-9}
2. Beta emitters, μc/mL	10^{-8}	10^{-8}
Bacteriological quality		
1. MPN index of coliform bacteria	Should be zero or less than 1	10 per 100 mL **

* Figures in excess of the permissive while not acceptable may still be tolerated in the absence of alternative and better sources, but up to the limits designated, above which the supply will not be acceptable.

** Occasionally, the samples may show an MPN index 3 to 10 per 100 mL provided this does not occur in consecutive samples. When consecutive samples show an MPN index exceeding 8 per 100 mL additional samples should be collected promptly from the sampling point and examined without delay. This should be done daily until the MPN index of samples collected on two successive days is within the acceptable limits. If necessary, samples should also be taken from several other points such as the service reservoirs, distribution systems pumping stations and treatment plant and examined for coliforms. In addition, the operation of all treatment process should be checked and remedial measures taken if necessary. When the results obtained over a period of one month are considered, not more than 10% of the samples examined during the period should have shown an MPN index of coliforms greater than 1 per 100 mL.

APPENDIX–III. MPN TABLE

No. of tubes giving positive reaction out of			*MPN index per 100 mL*	*95% confidence limits*	
3 of 10 mL each	*3 of 1mL each*	*3 of 0.1 mL each*		*Lower*	*Upper*
0	0	0	<1		
0	0	1	3	<0.5	9
0	1	0	3	<0.5	12
1	0	0	4	<0.5	20
1	0	1	7	1.0	21
1	1	0	7	1.0	23
1	1	1	11	3.0	36
1	1	0	11	3.0	36
2	0	0	9	1.0	36
2	0	1	14	3.0	37
2	1	0	15	3.0	44
2	1	1	20	7.0	82
2	2	0	21	4.0	47
2	2	1	28	10.0	150
3	0	0	23	4.0	120
3	0	1	39	7.0	130
3	0	2	64	15.0	380
3	1	0	43	7.0	210
3	1	1	75	14.0	230
3	1	2	120	30.0	380
3	2	0	93	15.0	380
3	2	1	150	30.0	440
3	2	2	210	35.0	470
3	3	0	240	36.0	1300
3	3	1	460	71.0	2400
3	3	2	1100	150.0	4800
3	3	3	>2400		

1. If instead of portions of 10, 1 and 0.1 mL, a combination of 100,10 and 1 mL is used then the MPN is recorded as 0.1 times the value given in the table.
2. For 1, 0.1 and 0.01 combination then 10 times the value in the table should be used.
3. For 0.1, 0.01 and 0.001 combination, 100 times the value given in the table should be used.