M.Sc. Final Year
Chemistry, Group-(D) Elective Paper
Paper I

ANALYTICAL CHEMISTRY

मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल
MADHYA PRADESH BHOJ (OPEN) UNIVERSITY – BHOPAL

COURSE WRITERS

Dr. Brajendra Singh Chauhan, Professor and Dean, Greater Noida Institute of Technology (G.N.I.T.), Greater Noida
Units (1.0-1.1, 1.2, 1.3, 1.3.1, 1.3.3, 1.3.4, 2.0-2.1, 2.2-2.2.4, 2.2.5, 2.3.2, 2.4-2.8, 2.3, 3.3, 3.3.1, 3.4, 3.5.1-3.5.4, 3.5, 4.0,4.1, 4.3.3, 4.2, 4.3.2, 4.4-4.9, 4.10-4.14, 4.3, 4.3.1, 5.2, 5.2.1, 5.3, 5.7-5.9, 5.0-5.1, 5.5-5.5.1, 5.5.3-5.5.6, 5.5.7, 5.10-5.14, 5.6)

Dr. Vijay Kumar Jha, Lecturer, Department of Biochemistry, Netaji Subash Medical College and Hospital, Bihar
Units (1.4-1.5, 1.6-1.10, 2.3.1, 5.5.2)

Dr. Manpreet Kaur Rawal, Assistant Professor, Department of Microbiology, Gargi College, University of Delhi, Delhi
Units (3.0- 3.1, 3.2, 3.3.2, 3.6-3.10)

Dr. Sumera Amin, Faculty, Institute of Public Health and Hygiene, New Delhi
Unit (5.4-5.4.2)

Published by Registrar, MP Bhoj (Open) University, Bhopal in 2020

Vikas® is the registered trademark of Vikas® Publishing House Pvt. Ltd.

VIKAS® PUBLISHING HOUSE PVT. LTD.
E-28, Sector-8, Noida - 201301 (UP)
Phone: 0120-4078900 • Fax: 0120-4078999
Regd. Office: A-27, 2nd Floor, Mohan Co-operative Industrial Estate, New Delhi 1100 44
• Website: www.vikaspublishing.com • Email: helpline@vikaspublishing.com

SYLLABI-BOOK MAPPING TABLE

Analytical Chemistry

Syllabi	Mapping in Book
UNIT - I: **Introduction:** Role of analytical chemistry. Classification of analytical methods classical and instrumental. Types of a instrumental analysis. Selecting an analytical method. Neatness and cleanliness. Laboratory operations and practices. Analytical balance. Techniques of weighing, errors. Volumetric glassware-cleaning and calibration of glassware. Sample preparations - dissolution and decompositions. Gravimetric techniques. Selecting and handling of reagents. Laboratory notebooks. Safety in the analytical laboratory.	**Unit-1:** Analytical Chemistry: An Introduction **(Pages 3-48)**
UNIT - II: **Errors and Evaluation:** Definition of terms in mean and median. Precision-standard deviation, relative standard deviation. Accuracy-absolute error, relative error. Types of error in experimental data-determinate (systematic), indeterminate (or random) and gross. Sources of errors and the effects upon the analytical results. Methods for reporting analytical data. Statistical evaluation of data-indeterminate errors. The uses of statistics.	**Unit-2:** Errors and Evaluation **(Pages 49-59)**
UNIT - III: **Food Analysis:** Moisture, ash, crude protein, fat, crude fibre, carbohydrates, calcium, potassium, sodium and phosphate. Food adulteration-common adulterants in food, contamination of food stuffs. Microscopic examination of foods for adulterants. Pesticide analysis in food products. Extraction and purification of sample. HPLC. Gas chromatography for organophosphates. Thin-layer chromatography for identification of chlorinated pesticides in food products.	**Unit-3:** Food Analysis **(Pages 61-94)**
UNIT - IV: **Analysis of Water Pollution:** Origin of waste water, types, water pollutants and their effects. Sources of water pollution-domestic, industrial, agricultural soil and radioactive wastes as sources of pollution. Objectives of analysis-parameter for analysis-colour, turbidity, total solids, conductivity, acidity, alkalinity, hardness, chloride, sulphate, fluoride, silica, phosphates and different forms of nitrogen. Heavy metal pollution-public health significance of cadmium, chromium, copper, lead, zinc, manganese, mercury and arsenic. General survey of instrumental technique for the analysis of heavy metals in aqueous systems. Measurements of DO, BOD and COD. Pesticides as water pollutants and analysis. Water pollution laws and standards.	**Unit-4:** Analysis of Water Pollution **(Pages 95-158)**
UNIT - V: **Analysis of Soil, Fuel, Body Fluids and Drugs** (a) Analysis of soil: moisture, pH, total nitrogen, phosphorus, silica, lime, magnesia, manganese, sulphur and alkali salts. (b) Fuel analysis: solid, liquid and gas. Ultimate and proximate analysis, heating values, grading of coal. Liquid fuels-Flash point, aniline point, octane number and carbon residue. Gaseous fuels-producer gas and water gas-calorific value. (c) Clinical chemistry: Composition ot blood-collection ond preservation of samples. Clinical analysis. Serum electrolytes, blood glucose, blood urea nitrogen, uric acid, albumin, globulins, barbiturates, acid and alkaline phosphatases. Immunoassay: principles of Radio lmmuno Assay (RIA) and applications. The blood gas analysis - trace elements in the body. (d) Drug analysis: Narcotics and dangerous drugs. Classification of drugs. Screening by gas and thin-layer chromatography and spectrophotometric measurements.	**Unit-5:** Analysis of Soil, Fuel, Body Fluids and Drugs **(Pages 159-233)**

CONTENTS

INTRODUCTION

NOTES

Analytical chemistry is the branch of chemistry that analyses the chemical composition of materials and develops instruments for examining them. It comprises both wet lab chemistry and equipment use. It engages a number of scientific disciplines including chemistry, biochemistry, physics, mathematics, and engineering. Separations such as precipitation, extraction, and distillation are used in traditional qualitative procedures. Color, odour, melting point, boiling point, solubility, radioactivity, and reactivity can all be used to identify a substance.

An analysis provides chemical or physical information about a sample. In an analysis, we determine the identity, concentration, or properties of an analyte. To make this determination, we measure one or more of the analyte's chemical or physical properties. Food analysis is a very important branch of analytical chemistry that provides information about chemical composition, processing, quality control (QC) and contamination of foodstuffs, ensuring compliance with food and trade laws. The chemical analysis of water provides considerable insight into the health and working of lakes, rivers, oceans and groundwater. It helps to identify and quantify the chemical components and properties of water samples. Water chemistry has helped scientists to define the different currents and circulation of the world's oceans, improved their understanding of water's interactions with Earth's geologic materials, and given insight into the impact of human activities on water bodies. Soil analysis is a set of various chemical processes that determine the amount of available plant nutrients in the soil, but also the chemical, physical and biological soil properties important for plant nutrition, or 'soil health'. It is crucial for enhancing the quality and/or quantity of the grapes produced as well as soil management efficiency. The analysis of drugs and various biological fluids is an important criterion for the determination of the physiological performance of a drug.

The book *Analytical Chemistry* comprises five units. It includes the basic concepts of analytical chemistry. It discusses the importance and the concepts of food analysis and water analysis. It elaborates the analysis of soil, fuel, body fluids and drugs. This book has been written in the Self-Instructional Mode (SIM) wherein each unit begins with an Introduction to the topic followed by an outline of the Objectives. The detailed content is then presented in a simple and an organized manner, interspersed with Check Your Progress questions to test the understanding of the students. A Summary along with a list of Key Terms and a set of Self-Assessment Questions and Exercises is also provided at the end of each unit for effective recapitulation.

UNIT 1 ANALYTICAL CHEMISTRY: AN INTRODUCTION

NOTES

Structure

1.0 INTRODUCTION

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the object in question. Significant contributions to analytical chemistry include the development of systematic elemental analysis by Justus von Liebig and systematized organic analysis based on the specific reactions of functional groups. Analytical chemistry has broad applications to forensics, medicine, science and engineering. Fundamentally, the analytical chemistry studies and uses instruments and methods used to separate, identify, and quantify matter. In practice, separation, identification or quantification may constitute the entire analysis or be combined with another method(s). Qualitative analysis identifies analytes, while quantitative analysis determines the numerical amount or concentration.

Typically, the qualitative methods use separations, such as precipitation, extraction and distillation. Identification may be based on differences in colour, odour, melting point, boiling point, radioactivity or reactivity. Quantitative analysis uses mass or volume changes to quantify amount. Analytical chemistry is also focused on improvements in experimental designs, chemometrics, and the creation of new measurement tools. Characteristically, a qualitative analysis helps in determining the presence or absence of a particular compound, but not the mass

NOTES

or concentration. This unit explain the role of analytical chemistry along with the classification of analytical methods. It also discusses the types of instrumental analysis and techniques of weighing. In addition, it will describe volumetric glassware and sample preparations including dissolution and decompositions. Also, it will introduce gravimetric techniques, methods of selecting and handling of reagents along with the safety rules in analytical laboratory.

1.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the role of analytical chemistry
- Discuss the different types of analytical analysis analytical methods
- Explain various laboratory operations and practices
- Anaylse the techniques of weighing errors
- Explain volumetric glassware-cleaning and calibration of glassware and gravimetric techniques
- Understand the ways to select and handle reagents, laboratory notebooks and practice safety

1.2 INTRODUCTION OF ANALYTICAL CHEMISTRY

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both quantitatively and qualitatively. Most chemists routinely make qualitative and quantitative measurements. So some scientists suggest that analytical chemistry is not a separate branch of chemistry but is a simple application of chemical knowledge.

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements as compounds contained in them. It is the science of chemical identification and determination of the composition of substances and materials and their chemical structures. The objective of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with a certain accuracy.

1.2.1 Role of Analytical Chemistry

Analytical chemistry uses science and technology to solve practical problems. It is applied in all areas of science, industry and medicine. Some of its roles in various determinations are enumerated below:

(i) Concentrations of O_2 and CO_2 in blood samples

(ii) Quantities of hydrocarbons, NOx and CO in automobile

(iii) Quantitative measurements of ionized Ca in blood serum to diagnose parathyroid disease in humans.

(iv) Quantitative determination of N in different types of foods, protein content and thus their nutritional value.

(v) Analysis of steel during production of carbon, nickel and chromium to achieved a desired strength, hardness, corrosion resistance and ductility

The interdisciplinary nature of analytical chemistry makes it a vital tool in medical, industrial, government and academic laboratories throughout the world (Refer Figure 1.1).

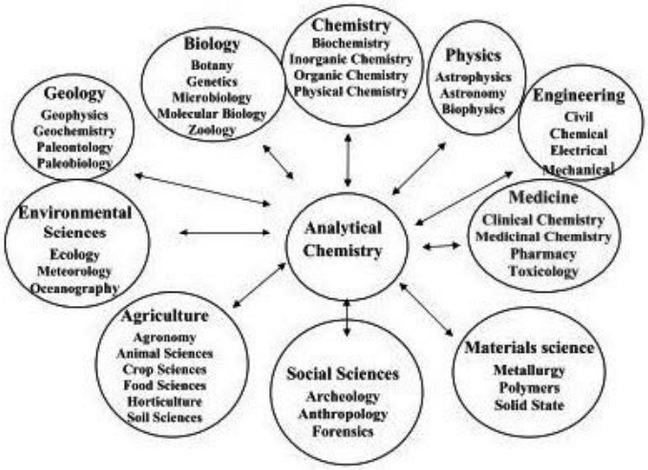


Fig. 1.1 *Relationship between Analytical Chemistry, Other Branches of Chemistry and the Other Sciences*

Analytical chemistry emphasized the development of great diversity of analytical methods and their various combinations with separation and concentration methods. These methods and their practical applications can be evaluated by studying their physicochemical and chemical nature, considering the general regularities that determine the procedure of measuring.

The importance of analytical chemistry in related scientific areas can be illustrated by considering its impact on clinical analysis and in pharmaceutical research and quality control. In the past, the diagnosis made by doctors were based on symptoms, clinical results obtained qualitatively and/or X-ray examinations. But later on sensitive chemical and instrumental tests were devised in order to detect abnormal and normal components of body fluids, since physiological diseases were accompanied by chemical changes taking place in the metabolic fluids. Furthermore, with intensive research it became possible to do quantitative analysis of abnormal and normal components of the body, and it became clear that these results could be used for diagnostic purposes.

Similarly, the quality of the manufactured drug in tablets, and solution and emulsion form must be carefully controlled in phamaceutical industry, otherwise the drug itself can affect the therapeutic value. In other pharmaceutical studies, it is important to establish the properties and therapeutic value of a drug before it is approved and made available to the public.

Analytical chemistry plays an extremely important role in the industry. Plants-producing metals require regular chemical analysis of the materials, intermediate compounds and finished products, as well as analytical control of the technological process. For example, ferrous metallurgy, poses a host of analytical tasks, such as control of minerals, concentrates, charge, molten steel, steels of various grades, refractory materials, fluxes, exhaust gases and sewage.

NOTES

Technological progress in any industry is impossible without the development and further improvement of analytical control of technological process. For example, in metallurgical industry, a rise in the production of metals and alloys and improvement in their quality greatly depend on the efficient work of analytical chemists in plants, mines and concentration mills and also on the development of methods for analysing raw materials, semi-finished and finished products.

Technical analysis is performed by chemical, physiochemical and physical analytical methods combined with chemical and physicochemical methods of separating and concentrating elements. Analytical methods in industry are worked out in field research institutes and plant laboratories for each type of material.

Depending on the material under analytical control and the aim of analysis, marking analysis and quick analysis are used. Marking analysis is carried out for controlling the chemical composition and properties of the raw materials and semi-processed materials that arrive at the plant. Marking analysers evaluate the quality of semi-finished and finished products and their compliance with the accepted standards. Quick analysis is used for the routine control of semi-finished and finished products. It helps in determining the validity of technology conditions. Volumetric, spectrophotometric and some physicochemical methods are preferentially used in quick analysis.

The analytical chemistry of rare elements plays an important role in geochemistry. For example, the differentiation in rock of rare elements having similar properties such as niobium and tantalum, zirconium and hafnium, tungsten and molybdenum, sulphur and selenium, rubidium and thallium, aluminium and gallium, nickel and cobalt, and radium and cadmium etc. is essential before accurate geochemical conclusion can be made. Traces of some rare elements such as molybdenum and vanadium are important in biochemical processes.

Electronic industry also requires high purity materials in the manufacturing of oscilloscopes, computers, radar apparatus, etc. Titanium of a very high purity is required for the manufacturing of cathode ray tubes and other evacuated apparatus. Thermo and photoelectric cathodes and cathode luminophores are produced from titanium, nickel, iron, tungsten, molybdenum, tantalum and niobium of very high purity.

1.2.2 Classification of Analytical Method: Classical and Instrumental

The final outcome of analytical chemistry is to provide information about the composition of a sample of matter. The classification of analytical methods depends upon different principles, but frequently these are classified into the following groups:

Chemical method (classical) of quantitative analysis

These methods are based on the course of chemical reactions and visual fixation of the property to be measured.

These methods make use of chemical reactions as a result of which an analytical element can be determined from the amount of reaction product or the volume of reagent used up in the reaction with a compound of the desired constituent. According to the quantity measured (mass, volume), chemical methods are classified as gravimetric and volumetric.

NOTES

Gravimetric methods depend on the conversion of the substance concerned to a reaction product and an accurate determination of its mass. Volumetric or titrimetric analysis involves consists in the measurement of the volume of reagent of exactly known concentration used up in the titration. The equivalence point should, however, be detected correctly. Equivalence point is the moment when the amount of standard solution added is equivalent to that of the substance being determined.

The equivalence point can be determined by the following:

(a) A change in the colour of the reactants themselves or indicators, which are introduced into the analytical solutions.

(b) A change in some physicochemical properties of the analyte (e.g., electrical conductivity), potential and optical density) measured instrumentally.

A sharp change in the colour of an indicator or in some property of the system indicates that the end point of the titration is reached. It should, however, be noted that the end point and equivalence point of the titration may not be identical because it depends on the following:

(a) Indicator used

(b) Properties of the titrant and the standard solution

(c) Properties of the reaction product.

There are various advantages of volumetric analysis. These include a wide variety of the types of chemical reactions that can be followed by titration methods, such as neutralisation reactions, oxidation reduction reactions, precipitation reactions, and complex formation reactions.

A direct titration is a more frequent technique where an unknown solution is titrated directly with a standard solution and the desired consituent is determined by measuring the volume of the standard solution required to react completely with the constituent.

When it is difficult to select an indicator for a direct titration or when the reaction is slow and does not involve a sharp change in concentration at the equivalence point back titration is used. In back titration, two standard solutions are employed. An accurately measured quantity of a standard solution is added in excess. After the completion of the reaction, the excess titrant is determined by titrating with another standard solution. The amount of the first standard solution that has been used up in the reaction with the desired constituent is determined by difference. For example, a measured amount of $CaCO_3$ is treated with an excess HCl standard solution, and an excess of HCl over that required to react with $CaCO_3$ is titrated with a standardised solution of NaOH.

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$$

$$HCl + NaOH \rightarrow NaCl + H_2O$$

Indirect methods of titration are used in those cases where a substance to be determined does not react directly with standardised solution or reacts with it in a non-equivalent amount. A substance under study, with the help of an auxiliary reagent, is converted to another chemical compound, which is formed in an amount equivalent to the content of the substance being determined. The new compound is titrated with a standard solution. For example, titration of $K_2Cr_2O_7$ with $Na_2S_2O_3$

NOTES

involves the reaction that fails to occur in equivalent amounts, because a part of $S_2O_3^{2-}$ ions is oxidised to $S_4O_6^{2-}$ ions and part to SO_4^{2-} ions. Moreover, difficulties also arise in selecting a suitable indicator. Thus, $K_2Cr_2O_7$ is first titrated with excess KI, as a result of which iodine evolved in an amount equivalent to the $K_2Cr_2O_7$ content is titrated with $Na_2S_2O_3$ standard solution using starch as an indicator.

$$Cr_2O_7^{2-} + 6I^- + 14H^+ \rightarrow 2Cr^{3+} + 3I_2 + 7H_2O$$

$$3I_2 + 6S_2O_3^{2-} \rightarrow 3S_4O_6^{2-} + 6I^-$$

Chemical methods of analysis are the basic methods generally used in analytical chemistry and are also known as the classical methods.

Advantages of chemical methods

Some of the advantages of chemical methods are as follow

(i) The procedure is accurate and simple.
(ii) The equipment required are cheap.
(iii) They are based on absolute measurements.
(iv) Specialized training is not required.

Disadvantages of chemical methods

Some of the disadvantages of chemical methods are as follows:

(i) Chemical environment is critical.
(ii) There is a versatility.
(iii) Accuracy decreases with decreasing amounts.
(iv) The procedure is time consuming.
(v) There is a specificity.

Instrumental Method Analysis

These methods include physicochemical and physical method of analysis and have greatly enhanced the scope of analytical chemistry in recent years. These methods are used to the same term, to avoid chemical separations or to obtain increased accuracy. The time saving features can be realised in routine analysis, or where a considerable number of determinations are to be made. The accuracy of some of the instrumental methods depends upon the accuracy with which the classical or wet chemical analysis can be made. In other words, we can say that an improvement in the classical methods of analysis will mean further improvement in the accuracy of instrumental methods of analysis.

Instrumental methods of analysis are based on the theory of relations between the content and the corresponding physiochemical and physical properties of the chemical system being analysed. Changes in the system properties are either detected or recorded through the measurement of current electrode potential, electrical conductivity, optical density, refractive index etc. with suitable and sensitive instruments.

Physicochemical methods may be direct or indirect, depending on the procedure of the determination. In direct methods, a substance is determined directly by measuring some property of the system. In indirect methods, a change in a property is used for detecting the end point of a chemical reaction, i.e., it

NOTES

serves as a peculiar sensitive indicator. The classification of physical and physicochemical methods generally depends upon the character of the measured properties of the system. These methods are generally classifed into two broad groups; spectral (optical) and electrochemical methods.

The optical methods are based on the relation between optical properties of a system and its composition. The electrochemical methods are based on the interdependence of electrochemical properties and composition of the system. In addition to these groups, radiometric, mass spectral and a number of other methods have also been used widely in quantitative analysis, and their number is fastly growing day by day. Physical and physicochemical methods are highly sensitive and rapid and therefore permit the determination of ultrasonic amounts of substances as little as 10^{-7} or even 10^{-9} percent.

Advantages of instrumental methods

Some of the advantages of instrumental methods are as follows:

(i) Small samples can be used.
(ii) High sensitivity is obtained.
(iii) Measurements obtained are reliable.
(iv) The determination is very fast.
(v) Complex samples can be handled easily.

Disadvantages of instrumental methods

Some of the disadvantages of instrumental methods are as follows:

(i) An initial or continuous calibration is required.
(ii) The sensitivity and accuracy depends on the instrument or wet chemical method.
(iii) The cost of equipment is high.
(iv) The concentration range is limited.
(v) Specialised training is needed.
(vi) Sizable space is required.

The method is of the following types:

(A) Electroanalytical methods: These methods involve the measurement of current, voltage or resistance in relation to the concentration of a certain species in solution. The techniques involved are as follows:

- Coulometry (measurement of current and time needed to complete an electrochemical reaction)
- Voltametry (measurement of current at a micro-electrode at a specified voltage)
- Potentiometry (measurement of potential of an electrode in equilibrium which an ion to be determined)
- Conductimetry (measurement of the electrical conductivity of a solution)

(B) Spectroscopic methods: These methods of analysis depend on the following:

- Measurmement of the amount of radiant energy of a particular wavelength absorbed or emitted by the sample
- Bending, scattering or delayed emission of radiant energy

NOTES

Table 1.1 *Classification of Instrumental Methods of Analysis*

S.No.	Method	Principle	Property measured
1.	**Electroanalytical methods**	Change in the electrical properties of the system.	Mass of deposited substance.
	(a) Electrogravimetry	Electrolysis is carried out.	Material deposited on one of the electrode is weighed
	(b) Coulometry	Deposition of matter on an electrode during electrolysis.	Quantity of electricity and time.
	(c) Conductimetry	Change in electrical conductivity of a solution during chemical reaction.	Electrical conductivity, Electrical resistance.
	(d) Amperometry	Potential applied between the indicator electrode and depolarized reference electrode is kept constant.	Current through the electrolytic cell is measured.
	(e) Potentiometry	Change in electrode potentials of a system during chemical reaction.	Electrode potential.
	(f) Polarography	Electrode polarisation.	Voltage, current.
2.	**Spectroscopic methods**	Interaction of matter with electromagnetic radiation.	Radiant energy of a particular wavelength.
	(a) Atomic: absorption spectroscopy	Atomising the specimen	Absorption of radiation.
	(b) Absorption spectrophotometry (colorimetry, photoelectro-colorimetry).	Absorption of poly and monochromatic radiant energy by molecules and ions in solution.	Optical density of the sooution.
	(c) Emission spectroscopy	Sample is subjected to an electric are or spark plasma. Emission of radiation.	Position and intensity of spectral lines.
	(d) X-ray spectroscopy	Emission of x-ray spectrum by atoms.	Position and intensity of spectral lines.
	(e) Raman spectroscopy	Absorption of monochromatic radiation by matter and emission of new radiation differing from that absorbed by the wavelength.	Same.
	(f) Turbidimetry	Absorption and scattering of a light beam by turbid media.	Amount of light stopped or scattered by a suspension.

	(g) Nephelometry	Reflection and scattering of a light beam by colloidal solution.	Same
	(h) Refractometry	Refraction of light by matter.	Refractive index
3.	**Mass spectroscopy**	Ionisation of atoms, ions and molecules by a combined action of electric and magnetic fields and appearance of mass spectra.	Position and intensity of signals in mass spectrum mass to charge ratio
4.	**Nuclear magnetic resonance**	Nuclear magnetism (resonance absorption of electromagnetic radiation by matter in magnetic field).	Position and intensity of lines of NMR spectrum
5.	**Radiometric methods**	Conversion of stable isotopes of an element to radio isotopes.	Intensity of radiation, induced radioactivity.
	Isotope dilution	Change in specific activity of the compounds labelled with a radioisotope	Radioactivity
6.	**Kinetic methods**	Speed of a chemical reaction may be increased by the addition of catalyst.	Concomiant change in the absorbance of solution for visible or UV radiation.
7.	**Thermal methods**	Recording as a function of temperature and time	Change in weight or energy.
	(a) Thermogravimetry, TG	Weighing of the substance while it is being heated.	Change in weight
	(b) Differential thermal analysis, DTA	Heat effects associated with physical and chemical changes of a substance are recorded when it is heated.	Difference in temperature.
	(c) Differential scanning colorimetry, DSC	Energy necessary to establish a zero temperature difference between a test substance and a reference material.	Change in energy

Spectroscopic methods are of following types:

(i) Absorption methods: These methods are classified according to the wavelength of light used as visible, ultraviolet or infrared spectrophotometry.

(ii) Emission methods: In these methods, a sample is subjected to heat or electrical treatment so that atoms are raised to excited states causing them to emit energy. The intensity of this energy is then measured. Some of the

NOTES

common emmision techniques are flame photometry emission spectroscopy, fluorimetry, etc.

(iii) **Nuclear magnetic resonance spectroscopy:** Nuclear magnetic resonance (NMR) spectroscopy is concerned with the study of interaction of energy with spin-active nuclei which have permanent magnetic moments and quantised nuclear spin states.

- **Electron spin resonance (ESR) spectroscopy:** Electrons in free radicals, atoms, ions or molecules (having unpaired electrons) change their spin under the influence of applied magnetic field and spectra arising is called ESR or EPR.

(iv) **Photoelectron spectroscopy (PES):** In PES, a beam of photons of known energy is allowed to fall on the sample and kinetic energy of the ejected electrons is measured. PES can be studied either using X-ray photons (XPES) or UV photons (UVPES).

(v) **Scattering methods:** Nephelometric and turbidimetric methods measure the amount of light stopped or scattered by a suspension.

C. X-Rays methods: These methods include the following:

(i) **Electron probe micro analysis:** Primary X-rays are produced when high speed electrons collide with a solid target. It is possible to identify certain emission peaks which are characteristic of elements contained in the target. The wavelengths of the peaks can be related to the atomic number of the elements producing them, so they provide a means of identifying elements present in the target sample. Further, under controlled conditions, the intensity of peaks may be used to determine the amounts of the various elements present. This forms the basis of electron probe micro analysis, in which a small target area of the sample is selected for identification.

(ii) **X-ray fluorescence:** When a beam of short wavelength primary X-rays strikes a solid target, it emits X-rays at wavelengths characteristic of the atoms involved. This is referred to as secondary or radiation. It is possible to get an idea of sample composition by examining the peak heights of the fluorescence radiation. X-ray fluorescence analysis is a rapid process which finds applications in metallurgical research, in processing of metallic ores and in the cement industry. Crystalline material will diffract a beam of X-rays. X-ray powder diffractometry can be used to identify components of mixtures.

D. Radioactive methods: Radioactive methods involve measuring the intensity of radiation from a naturally radioactive material and also measuring radioactivity induced by sample to a neutron source (activation analysis), or isotope dilution and radio immunoassay. Typical applications include determination of trace elements and quality control in semiconductor manufacturing.

E. Special Methods

(i) **Optical methods:** Refractometry can be used to measure the refractive index of liquids and to check the purity of the compound. In conjunction with a calibration curve, it can be employed to analyse a mixture of two

liquids. Optical rotatory dispersion and circular dichroism coupled to polarimetry correlate measurement of optical rotation and absorption.

(ii) **Kinetic methods:** Kinetic methods are based on the fact that the speed of a given chemical reaction may be increased by the addition of a small amount of catalyst, and within limits, the rate of catalysed reaction will be governed by the amount of catalyst present. If a calibration curve is prepared showing variation of reaction rate with the amount of catalyst used, then measurement of reaction rate will make it possible to determine how much catalyst has been added in certain instance. This provides a sensitive method for determining sub-microgram amounts of appropriate substances.

1.2.3 Selecting an Analytical Method

To choose the most appropriate method, analysts must be familiar with the practical details of the various techniques and theoritical principles on which they are based. They should consider the conditions under which each method is reliable, know possible interferences and finds ways to circumvent any problem. Analysys will be concerned with accuracy, precision, speed, sensitivity, selectivity availability of the instrument, level of analysis, time and cost of the procedure involved. Following points must be carefully considered for selecting an appropriate analytical methods:

- Type of analysis required; elemental or molecular, routine or occasional.
- Nature of the information which is sought.
- Size of the sample available.
- Problems arising from the nature of the material to be investigated, e.g., radioactive substances, corrosive substances substances affected by water.
- Possible interference from components of the material.
- Proportion of the constituent to be determined.
- The purpose for which the analytical data are required.
- Complexity of the material to be analysed.
- Concentration range which needs to be investigated.
- Accuracy
- Time required to complete the analysis.
- Number of analysis of the similar type which have to be performed.

There are a few steps involved in the quantitative analysis. In order to minimize error and to maintain accuracy and reproducibility, following steps must be considered carefully:

1. **Sampling:** Procedure employed for sampling depends on the size and physical nature of the sample.
2. **Preparation of Analytical Sample:** Analytical sample can be prepared by:
   (i) reduction in particle size,
   (ii) mixing for homogeneity,

NOTES

(iii) drying, and

(iv) determination of sample weight or volume.

3. **Dissolution of the sample:** It can be achieved by heating, ignition, fusion, solvents or dilution.
4. **Removal of interferences:** Interferences from the desired constituents can be removed by filtration, selective precipitation, masking, selective oxidation or reduction, solvent extraction, ion exchange, chromatographic separation (GLC, TLC, HPLC), etc.

   Note that samples of liquids and gases are frequently amenable to automatic treatment; most readily automated are the instrumental procedures for measurement, calibration, optimisation, statistical treatments and data presentation.
5. **Sample measurement and control of instrumental factors:** It can be achieved by standardisation, calibration, optimisation, measurement of response, absorbance, emission signal, potential and current.
6. **Employing spectroscopic investigations:** Spectroscopic investigations must be employed.
7. **Calculation of analytical results:** for the sample, statistical or chemometric evaluation of data.
8. **Presentation of data.** It can be done by print out, data plotting or storage (archiving).

Check Your Progress

1. What is the objective of analytical chemistry?
2. List the two types of analytical methods.
3. What is an equivalence point?
4. What can be measured by instrumental methods?

1.3 LABORATORY OPERATIONS AND PRACTICES

Let us study laboratory operations and practices in detail.

A. Personal protection

Some of the common personal practices are as follows:

- Work in the laboratory if, and only if, the teaching assistant is present.
- Work only on authorized experiments.
- Wear proper eye protection in the laboratory whenever any laboratory work is in progress.
- Wear shoes that do not have open spaces; sandals, flip-flops or any peep toe shoes are not acceptable.

NOTES

- Do not eat, drink or smoke in the laboratory. You must not even bring food or drink into the laboratory.
- Confine long hair and neckties. Loose jewelry may also be a hazard.
- Do not engage in acts of carelessness while in the laboratory.
- Work carefully with a full awareness of what you are doing in order to avoid ruining equipment or spilling chemicals.

B. Proper laboratory practices

Some of the common laboratary practices are as follows:

- Carefully read twice the label on a bottle before using its contents.
- Take only the quantity of reagent needed. NEVER return an unused reagent to its container.
- Mix reagents only when specifically directed to do so.
- Never place chemicals directly on the balance pan. Weigh reagents using a beaker, flask or weighing paper.
- If instructed to observe the odor of a chemical, do so by fanning air with your hand over the container toward your nose. Do not directly smell any substance.
- The fume hood is for your personal protection. You must leave the hood at the indicated working level for your protection and the protection of others. Do not lock the hood in the full-open position. The air-flow velocity is insufficient when the hood sash is in the fully-raised position.
- Never taste any reagent.
- Avoid handling chemicals directly with your hands. Protect your hands with gloves. If contact occurs, immediately flush the area with plenty of water.
- Use a bulb or a pipetting device to draw liquids into a pipette. Never do pipetting by sucking with your mouth.
- When diluting strong acids or strong bases, the acid or base should be added to the water, not the other way around.
- Try to avoid using heat guns but before turning it on, make always sure no flammable liquids or vapours are close in the area.
- Heat test tubes at the surface of the liquid. Agitate the tube. Be sure to slant its open end away from yourself and other people.
- Stay clear of an open vessel in which a process is occurring that could produce spattering.
- Keep reagents and equipment away from the edge of the lab bench.
- Do not use cracked glassware, as it may break when even slightly stressed.

1.3.1 Selecting and Handling of Reagents

Some of the common reagents used in qualitative analysis are as follows:

- HCl
- NH_3

NOTES

- $(NH_4)_2CO_3$
- NaOH
- K_2CrO_4
- $Na_2S_2O_3$
- KI
- $FeSO_4$
- $SnCl_2$
- HOAc
- Na_2CO_3
- $MgNO_3$
- $AgNO_3$
- $CuSO_4$
- $BaCl_2$
- $Pb(NO_3)_2$
- Ammonium Fluoride
- Ammonium Polysulphide
- Ammonium Acetate
- Ammonium Molybdate
- Potassium Ferrocyanide
- Mercuric Chloride

Reagents–Concentrated

- HCl
- H_2SO_4
- HNO_3
- NH_3
- H_2O_2
- HOAc

Reagents–Saturated

- Tartaric Acid
- $CaSO_4$ Solution
- Na_2HPO_4 Solution,
- Oxine Reagent (Mg)

Reagents–Solid

- NH_4Cl
- KNO_2
- $KClO_3$

NOTES

- PbO_2
- Sodium Sulphite
- Iron Filings
- Oxalic Acid
- Ammonium Nitrite
- Ammonium Acetate
- Ammonium or Potassium Thiocyanate
- Sodium Bismuthate

If you want to go for spot test then reagents are as follows:

- Nessler's Reagent (for Ammonium)
- Potassium Nitrite (Hg)
- K_2CrO_4 (Ag)
- $SnCl_2$ (W, Mo)
- Ammonium Sulphide (Pb)
- KI (Tl)
- Potassium Thiocyanate (Hg, Mo, Co)
- Thiourea (Bi, Se)
- Tartaric Acid (Cu)
- Cadion-2B (Cd)
- $AgNO_3$ (As)
- Hypophosphorus Acid (Te)
- Bismuth Nitrate (Sn)
- Rhodamine B (Sb)
- Catechol (Ti)
- Alizarin-S (Zr, Al)
- Anthranilic Acid (Ce)
- Potassium Ferrocyanide (Th, U)
- $CuSO_4$ (V)
- Diphenylcarbazide (Cr)
- Dimethylglyoxime (Ni)
- Sodium Bismuthate (Mn)
- Mercuric-Ammonium Thiocyanate (Zn)
- Sodium Rhodizonate (Ba, Sr)
- Picrolonic Acid (Ca)
- Magneson Reagent (Mg)
- Ferric Periodate Reagent (Li)

NOTES

Preparation of reagents

Some of the common reagents are prepared as follows:

- **Acetic acid (dilute):** Dilute 114 cm^3 of glacial acetic acid and add in to 1 dm^3 with distilled water.
- **Ammonium carbonate solutio:** Dissolve 96 g Ammonium Carbonate in distilled water and dilute to add into 1 dm^3 in water.
- **Ammonium fluoride Ssolution**: Dissolve 1 g NH_4F in 250 $cm^3$of distilled water.
- **Ammonium molybdate solution:** Dissolve 11 g crystalline amm. molybdate in a mixture of 15 cm^3 conc. NH_3 and 10 cm^3 water. Add 30 g amm. nitrate and after complete dissolution and dilute to 250 cm^3 with distilled water.
- **Ammonium polysulphide solution:** Dilute 140 cm^3 of conc. NH_3 solution with distilled water to 1 dm^3 and divide into two equal portions. Saturate one portion with H_2S gas (use fume cupboard!). Then add the second portion and mix. Add 32 g sulphur and heat gently until dissolved to give a yellow solution.
- **Ammonia solution (Dilute):** Dilute 140 cm^3 of concentrated NH_3 solution to 1 dm^3 with distilled water.
- **Copper sulphate solution:** Dissolve 15 g $CuSO_4.5H_2O$ in to 250 cm^3 of distilled water.
- **Ferrous sulphate solution**: Add 5 drops of conc. H_2SO_4 in 250 cm^3 of distilled water and dissolve 35 g $FeSO_4.7H_2O$ in it.
- **Lead nitrate solution:** Dissolve 20 g $PbNO_3$ in to 250 cm^3 of distilled water.
- **Potassium ferrocyanide solution**: Dissolve 2.5 g $K_4Fe(CN)_6.3H_2O$ in 250 cm^3 of distilled water.
- **Potassium Chromate Solution -** Dissolve 20 g K_2CrO_4 in 1 dm^3 of distilled water
- **Potassium iodide solution:** Dissolve 8 g KI in 250 cm^3 of distilled water.
- **Silver nitrate solution:** Dissolve 5 g $AgNO_3$ in 250 cm^3 of distilled water. (dark bottle).
- **Sodium carbonate solution:** Dissolve 13 g anhydrous Na_2CO_3 in 250 cm^3 of distilled water.
- **Sodium hydroxide solution:** Dissolve 20 g NaOH pellets in 250 cm^3 of distilled water.
- **Sodium thiosulphate solution:** Dissolve 6 g $Na_2S_2O_3.5H_2O$ in 250 cm^3 of distilled water.
- **Stannous chloride solution:** Add 25 cm^3 of conc. HCl to 25 cm^3 of distilled water, mix and cool. Dissolve 14 g $SnCl_2.2H_2O$ and dilute to 250 cm^3. Place a granule of tin metal at the bottom of the bottle to prevent oxidation.

1.3.2 Laboratory Notebook

NOTES

A laboratory notebook (often known as a lab book or lab notebook) is a primary research record. A lab notebook is used by researchers to keep track of their hypotheses, experiments, and preliminary analysis or interpretation of those experiments.

The laboratory notebook provides a personal record for documenting the progress of the experiment. While, the laboratory report serves a quite different purpose. It communicates your experimental work to other people. This requires a different style and approach.

All 'real' scientific work of any value eventually finds an expression in a written report. In the industrial research and development, reports communicate the supervisors and directors, they may circulate internally within the company and may even reach the other scientists working in the same field around the world. Some reports even get published in technical and scientific journals. Even technicians sometimes write such reports.

Many scientists or engineers discover the hard way that people judge the quality of experimental work by the quality of the reports. Ineffective reports might lead people to ignore the research itself and, on a very practical level, it might jeopardize the funding of that research as well.

Choosing a Notebook

For most of the purposes you might select a bound notebook, quadrille-ruled. A teaching lab might need tear-out duplicate pages for making carbon copies. An engineering or industrial research/development lab most likely requires a specific type notebook with pre-numbered pages and places for date and investigator's and supervisor's signatures on each page. It might be impossible to tear out a page without leaving any evidence. It is safe to select something which is clearly labeled as a laboratory notebook.

Preparing the notebook

A ball point pen should be used for the all entries, so that the marks will not smear nor will they be erased. Put your name, a telephone number and/or address and project name or course number on the front cover of the record. Put the same information on the first page inside, or on the inside front cover. If your notebook does not include a prelabeled table of contents section, reserve the next several pages for a table of content by labeling the top of each page as 'table of content' and numbering it. If your notebook does not have pre-numbered pages, you may wish to use lower case roman numerals, as is the rule in standard publication. Next, number the next several pages with Arabic numerals in sequence and you are ready to begin recording the data.

What to Enter

It is important that you enter all the procedures and data directly into your notebook in a proper manner, i.e., while you are conducting the actual work, your entries should be sufficiently detailed so that you or someone else could conduct any procedure with only

NOTES

the notebook as a guide. Very few students (and not that many researchers) record such sufficiently detailed and organized information. The most logical way of organizing notebook entries is chronological. If a proper chronological record is kept and co-signed by a co-worker or supervisor, then, it is a legally valid record. Such a record is necessary if you or your employer have keep your rights to your discoveries.

Depending on the requirements that are set by a teacher, supervisor, company, or whatever, it might not be necessary for you to confine your notebook entries to lab notes only. In contrast, a student might record his class lecture notes, lab lecture notes, ideas, questions, library research notes and notes that are part of any pre-lab preparation. The minimum entries for an academic lab course, for each lab study, should include title of the lab study, introduction and objectives, detailed procedures and data (recorded in the lab itself), summary.

You generally record a lot more information in a laboratory notebook than you would report in a research paper. For example, in a published article you do not report centrifuge type, rpm, rotor type or which machine was used. However, if a procedure is unsuccessful you may want to see that wether you used the correct rpm or correct rotor. Perhaps, the centrifuge itself was mis-calibrated. You will also need to know which machine you used. In a research paper, one does not reveal who performed what task, as such an information is useless to a third party. However, in the notebook, it is important to note who was responsible for what. Again, you may need such information for troubleshooting your experiments.

Making Entries

Someone else might need to consult your notebook, so please make your entries clear and legible way. When you make your first entries of the day, start by entering the date, in a month or abbreviation for the month (e.g., 5 Apr ’04, or April 5, 2004, but not 4/5/04).

The use of numerals will only enhance the confusion. For example, in Europe the day comes before the month. Thus, April 5, 2004 would be written as 5/4/04. When you start each new page of a notebook, enter the date next to the page number. Each page should be used only with the permission of the author. It should be numbered and dated consistently. Most of us use the upper right corner of each page for date and page number.

Depending on the design of your notebook, you may choose whether or not to use the back pages. If you leave them blank, you should put a corner-to-corner line through them to void all blank spaces. Some people even use the back for rough calculations; in that case, void remains a blank space. You might also decide to save space (and trees) and use both the sides of each page. Obviously, you cannot use both sides with notebooks that are designed for making duplicate copies. In situations where you turn in the duplicate copies to a supervisor, you obviously must start each new set of entries on a new page.

Write a title for each and every set of entries. Distinct sets of entries should be separated by using informative headings and by leaving a single space or two between the individual sets of entries. Specific information can be more readily

located that way. For a new laboratory study, write down a short introduction to the study and list its objectives. If you have a specific hypothesis, write it down. The object is to make it completely clear what do you intend to do. Record everything that you do in the lab, even if you are following a published procedure. For example, if you started by obtaining a quantity of tissue from an instructor, write it down describe it, note how much, what condition, etc. How much you write down is up to you, but each and every relevant information should be included in it. For example, it does not matter much if you received a chunk of liver in a red ice bucket or a black one. However, it does matter that the material was on ice. If you change a protocol in any way or decide between alternative methods, the correct information should be recorded in the notebook. For example, a protocol for tissue fractionation may recommend centrifugation at 9400 x g, but you may decide to use 12,000 x g in the lab. The correct g force must be noted.

If you make a mistake, cut it out and write the new information next to it. Never erase or obliterate an entry. When you finish a page, put a corner-to-corner line through any blank parts that could still be used for data entry. Every bit of every page must be legible and filled, either with information, or with a mark that voids particular section.

Who should record

- Any employee performing official-duty research.
- Anyone conducting research in a lab.
- Anyone using lab resources to perform research.

How to record

- **Binder.** Get a bound notebook, with numbered pages. Preferably, every other page should be detachable carbon paper, so that you can keep a copy of your handwritten notes in a separate location. If not, photocopy your notes regularly. Do not use a loose leaf or spiral notebook.
- **Record.** Write in ink, legibly. At least once in every binder, make sure every acronym, trade name, code, or jargon is defined, so that your reference is understandable by someone not working on your project.
- **Supplement.** Save all loose notes, e-mail messages and letters containing any part of the conception of an idea that could become an invention. Permanently affix (staple, tape, etc.) the copies of these into your notebook as you go, to maintain the chronological order as best as possible. Separately sign each such attachment so that part of your signature is on the attachment and part on the page (this is in addition to signing and dating each page).
- **Never.** Never blot out or erase mistakes – merely strike through them, put a brief note in the margin indicating why the material is stricken and keep going. Corrections risk making your work illegible, or worse, vulnerable to the accusation the better practice is to rewrite from scratch. Never rip out pages.
- **Sign and Date** every page. Always. If other people record data in your notebook, they too should sign and date each such page. If you modify or supplement anything afterwards, initial and date each such modification as well.

NOTES

- **Witness.** This also is crucial. Have at least one person (two if possible)– who is familiar enough with your field to understand what you are doing, but who is not directly involved in the research itself – sign and date each page as a witness that you did the work recorded ('Witnessed by Dr. Rahul Ranjan, / s/ , on _____, 2010'). For every page on which you write down a key insight, or solve a major problem, try to find two witnesses. If your work is not witnessed, your efforts at recording will be given little credence.
- **Electronic Records.** Computer software packages present many excellent research tools, but you should not rely on electronic records for the purposes of documentation. If you produce electronically anything you need to record, make a paper copy and affix it permanently in your notebook – signed, witnessed and dated, as always.
- **Storage.** Keep the original lab notebook in your lab; keep a copy in another building under lock and key. Your lab books and notes are government property.

What to record

- **Your Data.** Obviously, you have to record your data. But it also includes charts, graphs and figures you relied on and bibliographic references to key articles you used to design your experiments. Remember to affix these permanently. If you acquired materials from another lab, write that down as well.
- **Your Thoughts.** Feel free to incorporate any ideas, future plans, brainstorming activities, or wild speculations. An inventor is defined as a person who first conceives of the invention – this is your chance to show who thought of it and when. Avoid using words like 'obvious' and 'abandoned', because these words happen to havetremendous legal significance and if the words are applied inappropriately in your notes, they can be used against you.
- **Your Understanding.** Make sure everything you include can be understood by a colleague of 'ordinary skill' in your field. Your goal should be to make your records sufficiently complete and clear that such a colleague, reading only your notebook (and any articles cited in it), could duplicate your copies. Never skip pages, or leave large blank spaces; a blank page suggests that the work may not have been recorded chronologically.

Table of Contents

Record all the entries in the table of contents as you go along. You can organize it in anyway you want but it is advisable to include multiple levels in a table of contents, i.e., indicate where a new study starts and include the subheadings for specific parts of a study, methods, sets of data, etc. The idea is to enable someone (such a supervisor, grader or yourself a year from now) to find anything quickly. List every set of entries with dates and page numbers. If you are seriously anal-retentive, you might record every experiment in chronological order, then, use the remaining blank space to cross reference the contents experiment by experiment.

For a teaching lab, you might list all set of entries made in your notebook, in chronological order, including complete and informative titles. Examples of such sets of entries include an introduction, a summary, a set of procedures for a specific preparation, a complete data set, calculations for diluting samples or preparing assay standards. A grader should be able to find every specific entry quickly, without flipping through the pages.

Notebook checklist

As you record your activities in the laboratory, ask yourself, 'did you...'

- Keep up with the table of contents?
- Date each page?
- Number each page consecutively?
- Use continuation notes when ever necessary?
- Properly void all blank pages or portions of pages (front and back)?
- Enter all the necessary information directly into the notebook?
- Properly introduced and summarized each experiment?
- Include complete details of all first-time procedures?
- Include calculations?

1.3.3 Neatness and Cleanliness

The points that should be kept in mind to maintain neatness and cleanliness in the laboratary are as follows:

- Keep the working area of the laboratory as clean as possible. Clean up all spilled chemicals and dispose of chemicals according to the instructions given by teacher.
- Use distilled water for the preparation of all solutions.
- Clean the glassware with soap solution, but final rising of the equipment should be done with distilled water.
- Use the laboratory time well. The principles and experimental procedure upon which the experiment is based should be made clear before entering the laboratory. Organize what has to be done before and not during the laboratory time.
- Practical jokes and horseplay must be avoided in the laboratory.
- Co-operation is a must and generally required in the laboratory.
- Be patient if you are not following any thing in the laboratory. Speed will come only through careful planning and experience. The laboratory work should be performed patiently and not exceesively fast in relation to the amount of experience, otherwise results may be poor and accidents may occur because of carelessness.
- Perform experiments in terms of concepts, accuracy, precision and error.
- Use common sense in interpretation of the experimental procedure, use of chemicals, and relationship to the other individuals in the laboratory.

NOTES

- Be prepared in case of emergency. Accidents may occur but do not cause them through your carelessness. Locate the fire existinguishers and other emergency equipment in the laboratory. Treat burns caused by hot objects and corrosive acids. This involves a through washing with water under a shower. Fast treatment is necessary if eyes are involved. The eyes should be protected from accidents by wearing safety glasses.
- Bring a laboratory note book daily in the laboratory and all data should be recorded while performing the exprement, not later. Do not commit the observation or other data to memory for later recording.

1.3.4 Safety in Analytical Laboratory

Some of the instruction and safety rules that must be followed in the laboratory are as follows:

- Make certain to peruse all fire caution and wellbeing signs and adhere to the guidelines in case of a mishap or crisis.
- Guarantee you are completely mindful of your facility's/building's clearing strategies.
- Ensure you know where your lab's wellbeing hardware—including medical aid kit(s), fire dousers, eye wash stations, and security showers—is found and how to legitimately utilize it.
- Open flares ought to never be utilized in the research facility except if you have consent from a qualified expert or lab in-charge.
- Ensure you know about where your lab's ways out and fire alerts are found.
- A zone of 36" distance across must be kept clear consistently around all fire sprinkler heads.
- On the off chance that there is a fire penetrate, make sure to switch off all electrical gears and close all compartments.
- Continuously work in legitimately ventilated zones.
- Try not to bite gum, drink, or eat while working in the lab.
- Lab dish sets ought to never be used as sustenance or refreshment holders.
- Each time you use crystal, make sure to check it for chips and splits. Request/ inform your lab in-charge of any harmed dish sets so it very well may be legitimately discarded.
- Never use lab gear that you are not affirmed or prepared by your administrator to work.
- In the event that an instrument or bit of gear falls flat amid use, or is not working appropriately, report the issue to an expert immediately. Never endeavor to fix a gear issue alone.
- On the off chance that you are the last individual to leave the lab, try to bolt every one of the entry ways and check and fix or close all start sources.
- Never leave a progressing test unattended.
- Never lift any dish sets, arrangements, or different sorts of device above eye level.
- Never smell or taste synthetics.

NOTES

- Do not pipette by mouth.
- Ensure you generally pursue the best possible strategies for arranging lab squander.
- Report all wounds, mishaps, and split hardware or glass immediately, regardless of whether the episode appears to be little or irrelevant.
- In the event that you have been harmed, immediately call out and be active or boisterous till you get help.
- In case of a compound sprinkling into your eye(s) or on your skin, instantly flush the influenced area(s) with running water for no less than 20 minutes.
- In the event that you see any risky conditions in the lab, let your lab in-charge know at the earliest.
- Continuously tie back hair that is jaw length or more.
- Ensure that free attire or dangling gems is anchored, or abstain from wearing it in any case.
- Never wear open-toed shoes in the lab. Footwear ought to cover the foot totally.
- Never wear shorts or skirts in the lab.
- When working with Bunsen burners, lit supports, matches, and so on, acrylic nails are not permitted.
- Try not to enable any dissolvable to come into contact with your skin.
- All synthetic substances ought to be unmistakably named with the name of the substance, its focus, the date it was received, its expiry and the name of the individual lab in-charge of it.
- Before expelling any of the substances from a compound container, read the name twice.
- Never take a greater number of synthetic substances from a jug than you requirement for your work.
- Try not to return unused synthetics to their unique holder.
- Synthetics or different materials ought to never be removed from the research center.
- Synthetic concoctions ought to never be blended in sink channels.
- Combustible and unpredictable synthetic substances should just be utilized in a smoke hood.
- In the event that a synthetic spill happens, tidy it up immediately.
- Guarantee that all concoction squander is discarded legitimately.
- Never pour synthetic concoctions that have been utilized once again into the stock compartment.
- Never tap flagons that are under vacuum.
- Synthetic substances ought to never be blended, estimated, or warmed before your face.
- Water ought not to be filled concentrated corrosive. Rather, empty corrosive gradually into water while mixing always. By and large, blending corrosive with water is exothermic.

NOTES

Check Your Progress

5. How is ammonium carbonate solution produced?
6. What is a laboratory notebook?
7. Write any two saturated reagents.

1.4 ANALYTICAL BALANCE

An analytical balance (also known as a lab balance) is a type of balance that is used to quantify small amounts of mass in the microgram range. The measuring pan of an analytical balance (0.1 mg resolution or better) is enclosed in a glass enclosure with doors to keep dust out and any air currents from interfering with the balance's performance. This enclosure is often known as draft shield.

1.4.1 Techniques of Weighing, Errors

Mass is an invariant property of matter. Gravimetry is the process used to measure the mass of substances. Weight is a function of mass under the influence of gravity as expressed by the following equation:

$$\text{Weight} = \text{Mass} \times \text{Gravity}$$

Two substances of equal weights subject to the same gravitational force have equal masses. The mass is determined by using a balance to compare the mass of an unknown with that of a known mass. The process is called *weighing*; an absolute standard with which masses are compared is called *standard weight*. The words mass and weight are used interchangeably in practice.

The classic form of balance is a beam poised on a knife-edge fulcrum with edge placed at the centre of gravity, with a pan hanging from each end of the beam and a rigid pointer hanging from the beam at the poise point. With the object to be weighed on the pan and weight of equal mass on the other pan, the pointer comes to rest at the equilibrium of the balance point between the extremes of the path of excursion. Therefore, the weight required to achieve equilibrium is equal to the weight of the substances being weighed.

Although the classic form of the balance is of great antiquity, modern balances— both mechanical and electrical continue to apply the principle of equilibrium in a variety of ingenious ways. More than one type of balance is required for clinical laboratory because there is a necessity to weigh, i.e. 2 kg of timed urine output and microgram amount of drugs for reference solution. Coarse balances of large capacity have a detection limit of 0.1 μg.

All balances require a vibration-free location. The more sensitive the balance, the more the protection required, not only from vibration but also from air current that can disturb the equilibrium between the weighed object and weight. The null point (zero set) of the balance must be known or kept adjusted. Scrupulous attention to cleanliness is essential. Chemical substances being weighed should never be placed in direct contact with the pans. Disposable plastic weighing boats come in various sizes and are convenient for weighing most chemicals. They have largely

replaced weighing bottles and weighing paper for most uses. Loose crystals of chemicals or liquids with corrosive vapours should not be permitted to remain on or around the immediate area of the pans. Good weighing technique for samples weighing less than 1 g calls for handling weight with forceps and weighed objects with suitable utensils to prevent deposition of moisture, oils or salts from an analyst's skin.

Types of Balances

A common balance is used to find out the mass of a substance by comparing it with known masses. The balances used are of three types:

1. Physical balances
2. Two-pan analytical balances
3. Monopan balances

(1) Physical balances

Physical balances are used for weighing substances where great accuracy is not required. These are useful for the preparation of qualitative reagents.

(2) Two-pan analytical balances

These are useful for the preparation of qualitative reagents. The weighing range can be from 0.001 to 100 g.

Components of analytical balances are as follows:

- A beam
- Knife edges, screw nuts
- Stirrups
- Scale pans
- A pointer
- Ivory scale
- Rigid supports
- Wooden platform with levelling screws
- Handle
- Central vertical pillar

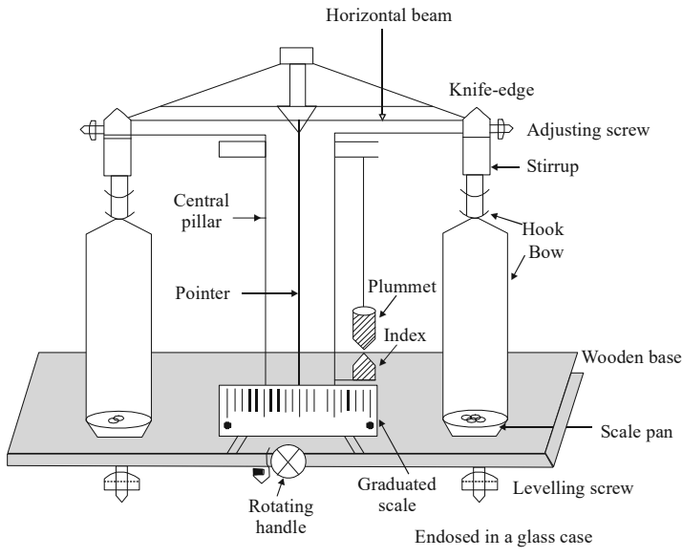
The **beam** is the sharp horizontal knife edge that supports the beam (made of metal) at the centre point. The **knife edge** rests on a metal pillar whose height can be adjusted using a handle. The **ivory scale** is found at the bottom of the **pillar** and has the pointer that swings across the scale. In other words, the pointer attached to the centre of the beam moves on this ivory scale. It is equally graduated and the graduated divisions are unnumbered but assumed to have 0 to 20 divisions continuously.

Scale pans, as the name suggests are the pans on which the objects to be weighed are placed. These are suspended from **stirrups** that rest on the edges of the knife of the beam. The balance as a whole rests on a **wooden platform** containing **levelling screws**. The pillar can be adjusted and made vertical using

these screws. Refer figure 1.2 (b). The balance is enclosed in a glass case as shown in Figure 1.2 (a).

NOTES

(a)



(b)

Fig. 1.2(a and b) *Two-Pan Analytical Balance enclosed in a Glass Case*

The weighing procedure of analytical balances is as follows:

- Adjust the levelling screw to make the pillar vertical.
- Release the beam by turning the handle. The pointer should show both sides equal. Otherwise, adjust it by turning the screw nuts at the end of the beam.
- Place the object in the left pan and the standard weight from weight bon, in the right pan.
- Adjust the weight from the weight box so that the pointer swings to the sides equally.

Points to be kept in mind while using analytical balance

Electronic analytical balances are also widely used. In fact, a single-pan (mono pan) electric balance is very convenient. While using an analytical balance, you should ensure that you:

- Use a balance that provides a sensitivity of at least 0.1 g at a load of 150 g, with appropriate weights.
- Use a balance having a sensitivity of 1 mg under a load of 10 g, for weighing small quantities (<2 g) of materials.

Figure 1.3 shows the various parts of an electrical analytical balance.

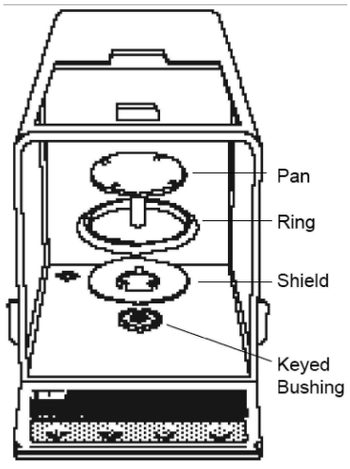


Fig. 1.3 *Components of Electrical Analytical Balance*

3. Monopan balances

The monopan balances are most sensitive and weighing accuracy can be obtained up to 0.001 mg. These balances are mainly used for the preparation of standard solution.

Components of monopan balances are as follows:

- A beam
- Central knife edge
- A pan
- Stirrups
- Series of calibrated weights
- A fixed constant counterweight

The weighing procedure of monopan balances is as follows:

- The beam of the single-pan substitution balance is situated asymmetrically on the central knife edge.
- The pan is supported by the stirrup placed on the outer knife edge.
- A series of calibrated weights are supported from the same end of the beam from which the pan is suspended.
- A fixed constant counterweight on the opposite end of the beam keeps the balance in equilibrium.
- The equilibrium is distributed when an unknown weight is placed on the weighing pan. The beam deflects to the direction of the heavier side.

NOTES

- To return the system to equilibrium, the corresponding mass of calibrated weight is removed from the front of the beam.
- The removed weight gives the weight of the unknown substance. This is called weighing by substitution.

Principle and Operations of Weighing

There are two operations used in weighing by direct comparison. Weights are added to one side of the beam to counterbalance the weight of the object on the other side. This approach is more common. In weighing by substitution, weights are removed from the side of a balance to which the object to be weighed has been added to restore equilibrium.

Two modes of weighing are used in practice:

1. Analytical weight is added to equal the weight of object being weighed.
2. Material to be weighed is added to a balance pan to achieve equilibrium with a preset weight.

The second mode is more commonly used in clinical laboratory, where the measure necessity is to weigh a fixed quantity of chemical so that a calibrator and unknown solution of known concentration may be prepared. Before weighing a sample or chemical, the weight of the container must be determined to subsequently allow deducting the weight of the container plus sample to obtain the net weight of sample. This is called taring. If taring process is impractical, the weight of empty container must be subtracted from the combined weight of container and the material to obtain the weight of the material alone. The sequence in weighing a sample using balance is as follows:

1. The balance is levelled by checking the level indicator and adjusting the feet.
2. Observe that the balance is not under direct sunlight and is in a draft-free location.
3. Set the balance to its zero point. If taring is used, set the read-out at zero. For the analytical balance, these settings are made with the sliding windows closed and the beam resting on the knife edge.
4. Look at the beam of the analytical balance. Open the window of the balance case and place the object to be weighed on the pan. Close the window.
5. Set the beam arrest knob in the intermediate position.
6. Make gross weight changes until the weight of the object is the range of the optical scale.
7. Fully release the beam and allow the pan to come to its final point of rest.
8. Record the mass of the object.

Care and Maintenance of Balances

- The beam of the balance should be arrested when not in use; also before adding or removing weight.

NOTES

- The weight should not be allowed to lie on the pan or in the wooden box.
- The balance should not be loaded with a weight greater than the maximum it is constructed to weigh.
- Clean the balances after use.
- Do not spill the chemicals on the scale pans.

Analytical Weight

The weight which is used to counterbalance the weight of object weighed on a two-pan balance and to verify the performance of both single- and two-pan balances. The National Institute of Standards and Technology (NIST) recognizes the five classes of analytical weights Class M, S, S-1, P and J:

- **Class M** weights are of primary standard quality which is used only to calibrate other weights.
- **Class S** weights are used for calibrating balances. In the clinical laboratory balance should be calibrated at least monthly and before conducting very accurate analytical work. Class S weights are typically made from brass or stainless steel and are lacquered or plated for protection.
- **Class S-1** weights have grater tolerance than the class S weights and are used for routine analytical work.
- **Class P** weights have tighter tolerance than any other class of weight.
- **Class J** weights are intended for micro analytical work and range from 0.05 to 50 mg. weighing by substitution.

Electrical Balances

The electrical balances (Figure 1.4) comprise three basic component systems: (1) a null detector, (2) a feed back loop of control the balancing force and (3) a read-out device.

Fig. 1.4 *Electrical Balance*

Performance and quality control of electrical balances

- The sensitivity is the ratio of the scale response change to the weight change. It is usually expressed as scale division deflection per mg.
- The precision of the instrument is expressed as standard deviation of repetitive measurement of the same mass.
- The accuracy can be checked by using national bureau of standard certified weights.

NOTES

1.5 VOLUMETRIC GLASSWARE

Volumetric glassware is chemistry glassware that has markings on it to facilitate measuring the volume of liquids easier. Beakers, Erlenmeyer flasks, graded cylinders, pipets, burets, and volumetric flasks are examples of volumetric glassware encountered in the chemistry lab. The graduated cylinder, volumetric flask, buret, and pipet are the four most popular types of volumetric glassware. These have special applications and will be examined separately.

Laboratory glassware means a variety of equipment, traditionally made of glass, and used for various scientific experiments in several departments of medical laboratories such as clinical chemistry, clinical microbiology, haematology and clinical pathology. In modern laboratories, some of the equipment is made up of plastics for cost saving, ruggedness and convenience in use. But glass is still used for some specifications like inertness, transparency, more heat resistance in comparison to plastic and relative ease for customization.

Borosilicate (formerly called pyrex) glass is often used to make laboratory glassware because it is less subject to thermal stress. In some specific applications, quartz glass is used because it has the ability to withstand high temperature and its transparency in certain parts of the electromagnetic spectrum. Dark glass bottles are used to keep out light so that the effect of light is minimized in relative solution. Glass equipment is used for some special purposes: to store chemicals which react with plastics; for example, hydrofluoric acid is stored and used in polythene containers since it reacts with glass.

Glassware is used for a variety of functions listed as follows:

- Volumetric measurement of liquid
- Storage of chemicals or samples
- Preparation of solutions or other mixtures
- Laboratory procedures like chemical reaction
- Heating, cooling and distillation
- Separation of biomolecules by chromatography
- Synthesis and growth of biological organisms
- Spectrophotometer procedure

The various types of equipment and instruments used in clinical biochemistry are as follows:

- Balances
- Hot plate and magnetic stirrers
- Centrifuges
- Hot air oven
- Incubators
- Water baths
- Photometers, nephelometers and spectrophotometers

NOTES

- pH meters
- Distillation plants and deionizers
- Automatic dispensers and diluters
- Autoanalysers
- Electrophoretic instrument
- Osmometers
- Electrolyte analysers: flame photometers and ion selective electrodes
- Acid-base analysers

Chemical Ingredients, Physical Properties of Laboratory Glassware

Laboratory glassware is usually manufactured from borosilicate glass, because it is resistant to the action of chemicals with the exception of hydrofluoric acid. It is made to withstand mechanical breakage and sudden changes of temperature. The gradients of borosilicate glass are shown in Table 1.2.

Table 1.2 *Chemical Properties of Glassware*

Name of Ingradient	Percentage Composition (%)
Silica (SiO_2)	80.6
Boric oxide (B_2O_2)	12.6
Sodium oxide (Na_2O)	4.15
Aluminium oxide (Al_2O_3)	2.2

Types of Volumetric Glassware

Specifications of volumetric glassware are A, B and student grade. The tolerances for accuracy of class glassware meet or exceed the strict requirements specified by the nbs in circular c-602. Only class volumetric glassware is acceptable by the cap for use in an approved clinical laboratory. General properties of glass are summarized in Table 1.3.

Serialized/certified flask: They are calibrated to class A specification. Each flask has an individual serial number and is furnished with a certification of identification which is traceable to NIST standards.

Verified class A flasks: These are manufactured using a process that validates the measurement of each individual flask. Each flask is labeled as verified and has a lot numbering enabling you to access a certificate of analysis from ASTM with lot-specific data regarding both measurement and methods.

Class A flask: These are manufactured to tolerances established by ASTM for volumetric ware. Utilize the same tolerances as certified and verified flask, but are not individually certified. Lot specific certification of compliance is available.

NOTES

Class B flask: Class B flask is generally calibrated twice the tolerance of class A flask.

Other types: There are also some specifications of other calibrated glassware set by various federal bureaus or professional societies.

Table 1.3 *General Properties of Glass*

Properties	Specification
ASTM class	A
Strain point	510°C
Annealing point	560°C
Softening point	821°C
Light protection	None

1.5.1 Cleaning of Glassware

Observe the following rules to handle glassware carefully:

- Hold beakers, bottles and flasks by the side and bottoms rather than by the top. The rim of beakers or neck of bottles and flasks may break if used as lifting points. Be especially careful with multiple-neck flasks.
- Do not tighten clamps.
- Use coated clamps for the prevention of glass to metal contact to avoid breakage while clamping glassware, and do not use excessive force to tighten clamps.

Glassware is coated with a tough and transparent plastic called vinyl. The coating, which is applied to the outside of the vessel, helps prevent exterior surface abrasion. It also causes minimization of the loss of contents and helps contain glass fragments if the glass vessel is broken. Attention to a few details regarding the use and care of glassware will maximize the life of glassware and will provide you with a safer laboratory.

Exposure to Heating and Cooling

Always watch evaporation work closely. A vessel, heated after evaporation has already occurred, may crack. Certain rules are listed here:

- Never put hot glassware on cold or wet surfaces, or cold glassware on hot surfaces; it may break with temperature change.
- Never heat glassware that is etched, cracked, chipped, nicked and scratched. It might break easily.
- Thick-walled glassware, such as bottles and jars, should not be heated over a direct flame or comparable heat source.
- Never heat glassware directly on electrical heating elements. Excessive stress would be induced in the glass, and this may result in breakage.

NOTES

- Never look down into any vessels being heated. A reaction may cause contents to be ejected.
- All glassware should be cooled slowly to prevent breakage.
- Observe extra care when removing glassware, particularly bottles, from ultra-low-temperature freezers to prevent thermal shock and cracking.
- For best results, immediately rinse the entire bottle under cold running water until thawing begins. Never place bottles directly from the freezer into warm water bottles.

Bunsen burners

Bunsen burners should be adjusted to get a large soft flame. These burners provide heat gradually but also more uniformly. Uniform heating is a critical factor for some chemical reactions. The ring stand or clamp holding the glassware should be adjusted such that the flame touches the glass below the liquid level. Heating above the liquid level does not help the heating of the solution and might cause thermal shock and breakage of the glassware.

Hot plate magnetic stirrer

There are various types of hot plates which are either heated by electricity or water. They may have a ceramic or metal top. You should must read the instruction manual before using a hot plate. On hot plates, the entire top surface heats and then remains warm for some time after you have turned it off.

When using electrically heated hot plates, be sure to check the wiring and the connector plug for wear. Always use a hot plate bigger than the bottom of the vessel being heated. Jars, bottles, cylinders and filter flasks, like thick wall containers, never are heated on hot plates.

Principle of operating: The instrument is based on the principle of rotating magnetic fields produced in a metal plate. When a magnet coated with Teflon is introduced in the magnetic field, it rotates and the chemicals get mixed up with the solvent.

Components of a hot plate

The components of a hot plate are as follows:

- Hot plate
- Magnetic stirrer
- Control for temperature
- Control for magnetic rotation speed
- Pilot lamp

Operation of the instrument

A hot plate is operated as follows:

- Put the beaker or conical flask containing distilled water (solvent) on the plate.
- Add the chemicals required for the preparation of the reagent and put the magnetic stirring paddle in the container.

NOTES

- Put on the main switch and by using the speed control knob, start the rotations of the paddle.
- Bring the speed control knob to zero position when the chemicals are completely dissolved in the solvent.
- Put off the main switch and make the final volume of the reagent by using required amount of the solvent.

Exposure to chemicals: The coating of glassware is specially designed. It has the capacity to resist leakage consequent upon a brief chemical exposure that may occur if the vessels are broken. It is advisable to avoid prolonged or repeated chemical exposure of the coating to aldehyde, ketones and concentrated acid.

Exposure to ultraviolet: Prolonged and repeated exposure of the glassware coating to direct sunlight or ultraviolet source is not recommended.

Exposure to microwave: Glassware is totally microwave safe. However, as with any microwave vessels, make sure there is water or other absorbing material in the microwave oven. Also, make sure all caps and closures are loosened.

Exposure to vacuum: All containers such as filter flasks and aspiratory bottles have exhibited the capacity to contain glass fragments upon implosion at room temperature. However, in following the safe laboratory practice, always use a safety shield around evacuated containers.

Autoclaving: Laboratory glassware must be sterilized utilizing liquid or dry cycle sterilization, which involves either no vacuum or low vacuum. Sterilization time should not be greater than 15 minutes at 121°C (250°F). Drying time should not be more than 15 minutes, 110°C (230°F). The actual cavity temperature of the autoclave ought to be checked to ensure that the temperature in the autoclave is not more than the temperature that is recommended for sterilization and drying.

Care and maintenance of centrifuge

- Place a centrifuge on a firm base; it should not be near the sensitive equipment e.g., photometer.
- The load must be balanced both by equal mass and by center of gravity across the center of rotation.
- The chamber should be kept clean. All spills should be cleaned immediately.
- Must make sure that the cover should be closed when operating centrifuge.
- Lubrication of bearing motor component, RPM verification and electrical wiring and timer verification should be done periodically.

Care and maintenance of incubator/hot air oven

- Do not forget to put off the main switch when the heating period is over.
- Clean the equipment after use.

A muffle furnace is a type of hot air oven. Temperature up to 800–900°C can be obtained by using a muffle furnace. In the determination of serum protein-bound iodine (PBI), for the conversion of organic iodine into inorganic iodine very high temperature is required. This experiment can be performed by using a muffle furnace. A thermostat is a bimetallic strip. Two strips of different metals of same

length are revetted together, length-wise, to form a themostat. On heating, the strip bends and the temperature remains constant.

NOTES

Care and maintenance of waterbath

- Before putting on the switch of the waterbath, make sure that it is sufficiently filled with water.
- Switch off the main switch after use.
- Cover the equipment after use.

Care and maintenance of colorimeters and photometers

- Always put a plastic cover on the equipment when not in use.
- Before putting the photometer on, put the filter and cuvette filled with distilled water in their respective position.
- Check the sensitivity of galvanometer occasionally by using a standard dichromate solution.

Care and maintenance of balances

- The beam of balance should be arrested when not in use.
- The weight should not lie on the pans.
- The weight greater than the maximum limit of balance should not be allowed.
- Do not weigh hot weight.
- Balance should be kept closed when reading is taken.

Calibration of glassware

According to the strictest of standards, every piece of volumetric glassware in the clinical laboratory should be coded and a record kept of its calibration. Any piece of glassware that does not meet class A tolerance should be rejected. To prepare a piece of glassware for calibration, rinse with tap water, followed by a thorough rinsing with reagent grade water.

Cleaning of Laboratory Glassware and Equipment

Cleaning is the removal of all foreign material (dirt and organic matter) from the object being processed. Glassware used in the general laboratory should be rinsed and immediately placed into a weak detergent solution. Corrosive chemicals should never be kept in glassware that may be spilled by unsuspecting personnel. Glassware must be rinsed, prewashed, washed, rinsed and finally rinsed with reagent-grade water before use. The surface of a thoroughly clean glass apparatus becomes uniformly wet, with no adhering water droplets. Special treatment is required in cases of stubborn grease and other organic residues. Let the glassware stand overnight in a sulfuric acid-dichromate mixture, prepared by pouring 1000-mLof concentrated sulfuric acid into 35-mLof saturated sodium dichromate. Avoid contact with the skin or clothing. Rinse the glassware thoroughly after removal from the mixture.

Bacteriologic glassware should be soaked in 2–4 per cent cresol solution, followed by autoclaving and a thorough washing procedure. Glassware used for

NOTES

iron determinations must be soaked in hydrochloric acid solution (concentrated HCl diluted 1:2) or nitric acid solution (concentrated -HNO_3 diluted 1:3) and then rinsed with reagent grade water.

Definitions in terms of laboratory equipment and glassware used are as follows:

- **Antimicrobial agent:** Any agent that kills or suppresses the growth or micro of ganisms.
- **Biological indicator:** A standardized preparation of bacterial spores on or in a carrier serving to demonstrate whether sterilizing conditions have been met.
- **Cleaning:** Cleaning is a process usually involving detergent or enzymatic process that removes foreign materials from an object. It is an essential step in reprocessing instrument and equipments.
- **Disinfection:** A process (physical or chemical) that destroys pathogens.
- **Disinfectant:** A chemical agent that destroys most pathogens but not bacterial spores.
- **Decontamination:** The use of physical or chemical means to remove, inactivate or destroy pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles. Decontamination could comprise cleaning, disinfection or sterilization as appropriate.
- **Sterilization:** A method that is used to remove and destroy all forms of microbial life including bacterial spores.

Laboratory equipment and glassware are devices that are essential for precise and accurate laboratory results; however, because they are typically designed for reuse, they can also transmit pathogens if any of the steps involved in reprocessing, cleaning, disinfection or sterilization are inadequate or experience failures.

Washing glassware should not ruin your chemical solutions or laboratory experiments. The problem is that the tolerance for chemicals varies with the kind of work you are doing, and some times a chemist does not know how important clean glassware is to an experiment till it has failed.

It is a common practice to clean all glassware before they are used. You may use any non-abrasive glassware detergent for hand or automatic dishwasher cleaning. You should take care to be sure the drying temperature does not exceed 110°C (230°F), if you are using dishwasher or glassware dryer. You should minimize exposure to dry heat.

There are two broad categories of clean glassware used in the chemistry laboratory: quantitative and normal. The most demanding application when a quantity is to be measured with a great precision require quantitatively clean glassware. At the precise level of cleanliness, there are none of the residues such as grease or other impurities left on the glassware. Usually clean glassware is free from most contaminants but some contaminants like grease are still there.

Health and safety consideration: A simple task such as washing glassware at the sink is potentially dangerous. You ought to wear eye protection, appropriate

for the task, at all times. Gloves should be put on even for usual cleaning, in case the glassware contains an irritant, lachrymator or toxic material. Make sure that any excess reagent has been disposed of in a proper manner and the vessels in which it was contained has been rinsed thrice into the waste container before cleaning. Triple rinsing does not mean that you fill vessel to the brim three times. This is wastage of solvent. To triple rinse a bottle or flask, simply place a small amount of solvent in the vessels and swirl to coat all of the inside surface. Then discard the wash in the appropriate wash bottle.

Cleaning Tips

The key to cleaning is doing it in a timely manner; letting dirty glassware sit for long periods of time guarantees a harder cleaning job.

- Disassemble your apparatus as soon as possible after you are finished with it. Remove all stopcocks and stoppers from addition funnels, separatory funnels and the like. Ground glass stopcocks and stoppers will freeze in place if certain reactants (e. g., bases) were used in them. Triple rinse all surfaces with an appropriate solvent to remove traces of solvents and reaction mixtures. Place the rinses in the appropriate waste container.
- Separate glassware that need to be quantitatively cleaned from that which do not. In this way, you do not waste time trying to quantitatively clean items that do not need to be.
- Generally, graduated cylinders, beakers, Erlenmeyer flasks, burettes and pipettes that were only used to dispense or store reagents only need to be triple rinsed with a compatible solvent followed by tap water and a final DI water rinse, if desired. Air dry on a drying rack. In some cases, you may need to be more thorough.
- Büchner funnels, etc. should be rinsed with an appropriate solvent to remove substances stuck to them. Running a solvent through them backwards using gravity (never use vacuum to speed up this process!) can help remove contamination from the inside of the funnel and from the surface of fritted funnels. Follow this by tap water and DI water rinses and air dry.

Cleaning Procedure

The following steps should be followed for glassware for which a simple solvent rinse is not sufficient. If you need quantitatively clean glassware, these should be the first steps toward this goal, and more aggressive cleaning methods may be required (vide infra).

- Degrease the ground glass joints of your glassware by wiping them with a paper towel soaked in a small amount of ether, acetone or other solvent (CAUTION! Wear appropriate gloves and be careful not to be exposed to the vapours).
- Place the glassware in a warm concentrated aqueous solution of Alconox, or other detergent, and let it sit for several minutes.
- Scrub the glassware but ensure that your brush is in good shape before scrubbing.

NOTES

- Use tap water to rinse thoroughly before giving a final rinse with DI water.
- If the glass is quantitatively clean, the water will sheet cleanly off the glass. In case the water does not sheet off the glass, and you want the glassware to be quantitatively clean, then soak it and scrub again as explained earlier. If you find that solid particles continue to cling to the glass, or if you spot some grease, you may have to take some aggressive action.

Aggressive Cleaning Techniques

The following cleaning techniques are two of the more commonly used methods of removing contaminants from glassware. These are usually used after normal cleaning has failed, and they are often used together, because each is effective at removing different types of contaminants. Care must be taken using either one because of the corrosive nature of the solutions used.

- If the contaminant is a metal-containing compound, soak the piece of glassware in a 6 M HCl solution. However, be very careful and ensure that you wear proper gloves, because this solution can cause severe burns. Wear appropriate gloves. Once the solid has dissolved, copiously rinse the item with tap water, and then repeat the general cleaning steps above. This technique will also remove some organic residues (not grease).

If the contaminant is organic, submerge the item in a base bath (a saturated NaOH or KOH solution in ethanol, methanol or isopropanol). Be careful because the base bath will dissolve skin, and alcohols are flammable! Wear butyl gloves and keep ignition sources away from the base bath. Be sure that the piece of glassware is completely filled with the solution and is sitting upright. After several minutes of soaking, carefully remove the item (it will be slippery), and rinse thoroughly. If the glassware is not quantitatively clean, the base bath may need to be soaked for a longer time and the general cleaning steps should be repeated.

Avoid soaking the following items in a base bath for long:

- Glassware contaminated with metal-containing compounds
- Glass fritted funnels
- Cuvettes
- Volumetric glassware (pipettes, volumetric flasks)
- Any glassware contaminated by an oxidizing agent

Anything that has not been washed according to the above steps first

Glass fritted funnels and volumetric glassware can be rinsed briefly with the base bath solution to remove small amounts of grease, but prolonged exposure to the caustic solution can damage these items.

Even More Aggressive Cleaning Methods

Sometimes 6 M HCl and a base bath are not sufficient, and even more aggressive techniques need to be employed. Please be warned that all these techniques will severely damage the eyes, skin, mucous membranes and lungs. You have to be cautious while using these methods. Wear butyl gloves (not latex or nitrile exam gloves), eye protection and a lab coat. Work in the hood.

NOTES

Undergraduate students should consult their supervisors before using these techniques, and they must be directly supervised by a faculty member at all times when using these methods (no exceptions).

Aqua Regia is an extremely powerful oxidizing solution prepared from 1 part concentrated HNO_3 and 3 parts concentrated HCl (it is recommended that 1 part H_2O be added if the aqua regia will be stored to minimize the generation of Cl_2). It is the only acidic solution that will dissolve gold and will oxidize just about everything else. Extreme caution must be used when working with aqua regia because it generates Cl_2 and NOx gases in addition to causing severe tissue damage. Clean the glassware before soaking in aqua regia and then rinse thoroughly with water.

Acidic Peroxide Solution can be most conveniently prepared by dissolving the commercially-available "NoChromix" mix in concentrated H_2SO_4. You need to follow the instructions on the package. Alternatively, you could prepare a solution by mixing equal proportions of concentrated H_2SO_4 and aqueous H_2O_2 solutions (remember to add the acid to the H_2O_2). A 3 per cent H_2O_2 solution is usually sufficient, and under no circumstances should H_2O_2 solutions greater than 10 per cent be used. The H_2O_2/ H_2SO_4 solution is both a strong oxidant and a strong reductant, so care should be taken during usage. Another acidic peroxide cleaning solution can be prepared by dissolving 36 gm $(NH_4)2S_2O_8$ (ammonium peroxydisulfate) in 2.2 L of 98 per cent H_2SO_4 (can be made right in the bottle of H_2SO_4, if the bottle is loosely stoppered). The procedure for these solutions is the same as for aqua regia as are the precautions for their use.

Chromic Acid is a solution of C_rO_3 in concentrated H_2SO_4. A premeasured mix is available under the name "Chromerge", which should be treated in the same way as aqua regia or acidic peroxide solutions. Because high-valent chromium is carcinogenic and teratogenic and causes severe environmental damage, the use of chromic acid is not recommended.

Hydrofluoric Acid Concentrated solutions of HF will remove just about everything from glass and will even etch the surface of the glass itself. It should not be used on calibrated volumetric. HF causes severe, painful burns that do not heal well, and prolonged or intense exposure can lead to a very slow or painful death. It is not to be used by any students at Truman under any circumstances.

Special Cases

Generally, **cuvettes o**nly need to be rinsed in the appropriate solvent and the outside wiped with a Kimwipe immediately after use. If some residue is found clinging to the a cuvette, it is best to soak it in solvent first and gently coax the solid off the side with a cotton swab. Never use a brush on a cuvette! If this fails, one of the acidic cleaning solutions mentioned above can be used (but never HF!). Use of a base bath on cuvettes is not recommended because it tends to etch glass surfaces.

Fritted funnels can be generally cleaned by inverting and allowing the solvent to flow by gravity through the frit in reverse. Use of vacuum is not recommended. Solvent can also be pulled through the frit (in the normal direction) under vacuum. Recalcitrant gunk can usually be removed by soaking in acid, followed by copious rinsing with water under vacuum. As HF and the base bath solution etch glass,

NOTES

they should not be used on fritted funnels (a brief exposure to a base bath is not usually fatal to a frit, but prolonged soaking can be).

Protein contamination can be removed by scrubbing with detergent, but occasionally protein defies removal. In such case, you can proceed to the more aggressive acidic solutions, or you can prepare a peptidase solution (an enzyme that degrades proteins). The enzymatic approach is a little slower than the forcing methods, but it is gentler and so can be used in situations where the contaminated item has low compatibility with acid.

Drying Glassware

Wet glassware can be dried by 1) placing it on the drying rack (or invert on a paper towel), 2) placing it in the drying oven (for items that are water-wet only, no flammable solvents) or 3) rinsing with a solvent such as acetone, methanol or ethanol and then gently blowing compressed air into the vessel until it is dry. The first method is preferred for drying quantitatively clean glassware (provided that the prongs of the drying rack are not inside the item, thus contaminating it). Volumetric glassware and cuvettes should never be placed in drying ovens, even if they are not quantitatively clean. The third method is acceptable only when the compressed air supply is known to be free oil and other contaminants. An alternative to blowing air into the item is to use an aspirator, or house vacuum, to pull air into the item.

Cleaning Procedure for Used Pipette Tips, Centrifuge Tubes, Nalgene Bottles and Savillex Beakers

Note: New centrifuge tubes and pipette tips should be cleaned separately!

1. Remove any markings and labels using ethanol on a paper tissue
2. Empty and rinse three times with tap water in a basin
3. Rinse in Milli-Q water in a large plastic container
4. Place in Milli-Q water in a large glass beaker for 24 hours.

Savillex beakers only:

- In the fumecupboard, add approximately 4–5 ml of concentrated HNO_3 to all savillex sample beakers, close lids tightly and place on hotplate for 24 hours.
- Remove beakers from hotplate and allow to cool before emptying.
- Place in 50:50 HNO_3:Milli-Q water cleaning solution in large glass beaker in fumecupboard for 24 hours.
- Place in Mili-Q water in large glass beaker for 24 hours.
- Rinse three times with Milli-Q water.
- Dry in oven at 60oC until dry.

NOTE: ALL CLEANING SOLUTIONS (MILLI-Q WATER AND 50:50 HNO3:MILLI-Q WATER) MUST BE REPLACED REGULARLY.

NOTES

Cleaning Procedure for New Pipette Tips and Centrifuge Tubes

Note: Used centrifuge tubes and pipette tips are cleaned separately!

- Rinse in Milli-Q water in large plastic container.
- Place in 50:50 HNO3:Milli-Q water cleaning solution in large glass beaker in fumecupboard for 24 hours.
- Place in Mili-Q water in large glass beaker for 24 hours.
- Rinse three times with Milli-Q water.
- Dry in oven at 60oC until dry.

NOTE: All Cleaning Solutions (Milli-Q Water and 50:50 HNO_3: Milli-Q Water) Should be Regularly Replaced)

Cleaning Procedure For All Glassware

- Using ethanol on a paper tissue, remove any markings and labels.
- Rinse three times with tap water in basin.
- Rinse three times with Milli-Q water.
- Rinse at least three times with 50:50 HNO3:Milli-Q water cleaning solution.
- Rinse three times with Mili-Q water.
- Allow to air-dry on the drying rack.

How to wash out common chemicals

Water-soluble solution: Rinse three to four times with deionized water, then put the glassware away (e.g. sodium chloride).

Water-insoluble solution: Rinse two to three times with ethanol or acetone, rinse three to four times with deionized water, then put the glassware away. In some situations, other solvents might be used for initial rinse (e.g. chloroforms).

Strong acids: Under the fume hood, carefully rinse the glassware with a lot of tap water. Rinse three to four times with deionized water and then put the glassware away (e.g. concentrated hydrochloric acid).

Strong base: Under the fume hood, carefully rinse the glassware with copious volumes of tap water, rinse three to four times with deionized water, then put the glassware away (e.g. sodium hydroxide).

Weak acid: Rinse three to four times with deionized water and put away the glassware (e.g. dilution of any strong acid).

Weak base: Rinse with tap water thoroughly to remove the base and then rinse with deionized water before putting away the glassware [e.g. sodium hydroxide (NAOH)].

After rinsing with deionized water, we must follow more aggressive cleaning methods discussed hereafter.

Caution

- All methods do severe damage to eye or skin. Medical technologists must wear eye protection shield, laboratory coat and gloves.

NOTES

- Entry level must check with their objective-level medical technologist before using these methods, and they must be under the direction of medical scientist or subject expert at all time when they using these method (no exception).
- Never soak following glassware in base bath for prolonged period :
  - o Glassware contaminated with metal containing compounds
  - o Glass-fitted funnels
  - o Cuvettes
  - o Pipette
  - o Any glassware contaminated with oxidizing agent

Aqua regia (branded dilution of 1 part HNO_3 and 3 parts HCl concentrated): This is an extremely powerful oxidizing solution. This is the only acidic solution which can dissolve gold and will oxidize just about everything else. Aqua regia generates Cl_2 and NOX gases, which must be use with extreme caution. Wash the glassware thoroughly with water after soaking in aqua regia.

Chromic acid (solution of CRO_3 in concentrated H_2SO_4): This solution should be treated as aqua regia solution because high valent chromium is carcinogenic, teratogenic and will cause severe environmental damage.

Hydrofluoric acid (HF): Concentrated HF will remove everything from glass and even the surface of glass itself also. Therefore, do not wash calibrated volumetric by this chemical. HF cause severe painful burn.

Special case

Certain special cases are discussed here.

Cuvettes: Generally you need to only rinse the cuvette in appropriate solution and wipe immediately after use. If something has adhered to a cuvette, soak the cuvette in solvent and gently coax the solid off the side with cotton swab. Never use brush for cuvette washing. Do not use HF solution for cuvette washing.

Protein contamination: Usually proteins are removed by use of detergent but occasionally protein defies removal. In that event, need use more aggressive acidic solutions or an enzymatic solution to degrade the protein. Enzymatic method approach is a bit slower than the forcing methods but it is relevant and gentler, so can be used when the contaminated items is incompatible with acid.

Drying glassware: After cleaning of glassware, place all on the drying rack or invert on a paper towel for drying, then placed in drying oven if glassware are water wet only. Volumetric glassware and cuvettes are never to be placed in drying ovens.

1.5.2 Calibration of Glassware

A known, specified density liquid and an analytical balance are routinely used to calibrate glassware. The process entails determining the amount of liquid that the glassware can contain, then dividing that mass by the density of the liquid to obtain the equivalent volume of liquid. Temperature affects density, therefore you'll need to check the temperature of the liquid and find up acceptable density figures. To different degrees of accuracy, all volumetric glassware is calibrated with marks

used to calculate a certain volume of liquid. The bottom of the curved surface of the liquid, the meniscus, should be situated at the scribed line for the desired volume to read this volume accurately. Filling these things (such as pipets) with the appropriate volume of liquid and then dispensing the liquid into a previously weighed container of appropriate size is the proper process. The volume is estimated after determining the mass of the dispensed liquid.

1.5.3 Sample Preparation-Dissolution and Decompositions

In analytical chemistry, sample preparation is the process of extracting a representative piece of material from a larger amount and preparing it for analysis. When it comes to analytical chemistry, sampling and sample preparation have a distinct meaning and significance. Solid-phase extraction, LC, dialysis, microwave sample preparation, flow injection analysis, and segmentation flow analysis are the major sample preparation techniques that can be automated.

This can be accomplished through a variety of methods, including digestion and leaching with concentrated or dilute acids, fusion with acid or alkaline fluxes, pyrolysis to liberate volatile compounds, and partial or selective extraction of constituents using a range of reagents.

Most procedures need the dissolving of a sample, which is required if separation/preconcentration techniques are performed. Acid attack, fusion, and breakdown in gas streams are the three major types of decomposition techniques. For the sake of speed, they are applied to powder samples (finer than 100 mesh). Metals, alloys, geological samples, and organic compounds are commonly dissolved using strong inorganic acids (HCl, HNO_3, $HCl0_4$, and H_2SO_4) and others (H_3PO_4 and HF). The fusion process is a heterogeneous reaction whose rate is limited by the contact surface, insoluble precipitate collection on the surface, and diffusion conditions.

The goal of sample dissolving is to quantitatively mix a solid or nonaqueous liquid sample with water or mineral acids to produce a homogeneous aqueous solution that can be separated and analysed later. The techniques include in sample dissolution are acid digestion, microwave digestion and fusion digestion.

Check Your Progress

8. Define Gravimetry.
9. What is volumetric glassware?

1.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. The objective of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with a certain accuracy.
2. The two methods are classical and instrumental.

3. Equivalence point is the moment when the amount of standard solution added is equivalent to that of the substance being determined.
4. The methods which measure an electrical property, absorption of radiation or the intensity of an emission, require the use of a suitable instrument, polarograph, spectrophotometer, etc. and in consequence are referred to as instrumental methods.
5. Dissolve 96 g ammonium carbonate in distilled water and dilute to add into 1 dm3 in water.
6. A laboratory notebook (sometimes known as a lab book or lab notebook) is a primary research record.
7. Saturated reagents are as follows:
   - Tartaric Acid
   - $CaSO_4$ Solution
   - Na_2HPO_4 Solution,
   - Oxine Reagent (Mg)

   (Write any two.)
8. Gravimetry is the process used to measure the mass of a substance.
9. Volumetric glassware is chemistry glassware that has markings on it to facilitate measuring the volume of liquids easier.

1.7 SUMMARY

- A laboratory notebook (often known as a lab book or lab notebook) is a primary research record.
- An analytical balance (also known as a lab balance) is a type of balance that is used to quantify small amounts of mass in the microgram range.
- There are majorly two weighing techniques, direct weighing and indirect weighing.
- Gravimetric analysis is a set of methods used in analytical chemistry to determine the mass of an analyte (the ion being studied) in order to quantify it.
- Volumetric glassware is chemistry glassware that has markings on it to facilitate measuring the volume of liquids easier. Beakers, Erlenmeyer flasks, graded cylinders, pipets, burets, and volumetric flasks are examples of volumetric glassware encountered in the chemistry lab.
- Unclean volumetric glassware must be cleansed and dried before use, as contamination mistakes might occur if it is not.
- A known, specified density liquid and an analytical balance are routinely used to calibrate glassware. The process entails determining the amount of liquid that the glassware can contain, then dividing that mass by the density of the liquid to obtain the equivalent volume of liquid.

- In analytical chemistry, sample preparation is the process of extracting a representative piece of material from a larger amount and preparing it for analysis.
- Acid attack, fusion, and breakdown in gas streams are the three major types of decomposition techniques.
- There must be various precaution that must be taken while performing laboratory operations and practices.
- Universal system of analysis of organic compounds includes preliminary tests, physical constants, analysis for elements present and solubility tests.

1.8 KEY TERMS

- **Classical Analysis:** It refers to analytical techniques that do not require the use of any mechanical or electrical devices other than a balance**.**
- **Instrumental Analysis:** It is a branch of analytical chemistry that uses scientific tools to analyse analytes.
- **Analytical Balance:** These are laboratory devices that are designed to precisely measure mass.
- **Laboratory Notebook:** It is a tamper-proof record of laboratory activities such as the usage of testing equipment, laboratory environmental conditions, and crucial standard and supply consumption.

1.9 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Differentiate between marking analysis and quick analysis.
2. State the advantages and disadvantages of the classical method of analysis.
3. What is the checklist while making a record of activitieas in the laboratory notebook.

Long-Answer Questions

1. Discuss in brief the relationship between analytical chemistry and other branches of chemistry and sciences.
2. Explain the two types of analytical methods.
3. Analyse various laboratory operations and practices.
4. Describe various safety rules in analytical laboratory.

1.10 FURTHER READING

Dash, Dhruba Charan. 2011. *Analytical Chemistry*. New Delhi: Prentice Hall India Pvt, Limited

NOTES

Hibbert, D.B. and J.J. Gooding. 2006. *Data Analysis for Chemistry*. UK: Oxford University Press.

Benwart, G. J. 1987. *Basic Food Microbiology*. New Delhi: CBS Publishers & Distributors.

Christian, Gary D. 2007. *Analytical Chemistry*, 6th Edition. Noida: Wiley India Pvt. Limited

UNIT 2 ERRORS AND EVALUATION

NOTES

Structure

2.0 INTRODUCTION

Any measurement is limited by the precision of the measuring instruments and the technique and the skill of the observer. Where a measurement consists of a single reading on a simple piece of laboratory equipment, for example a burette or a thermometer, one would expect the number of variables contributing to uncertainties in that measurement to be fewer than a measurement which is the result of a multi-step process consisting of two or more weight measurements, a titration and the use of a variety of reagents. It is important to be able to estimate the uncertainty in any measurement because not doing so leaves the investigator as ignorant as though there were no measurement at all. Analytical chemistry is dependent on repeatability, accuracy and reliability. In analytical chemistry, however, every measurement contains some degree of uncertainty, which is referred to as error. The difference between the experimental mean value and a true value is the error. This unit will discuss the concepts of mean, median and standard deviation. It will explain the types of error, their sources and their effects on analytical results. In addition, it will describe the methods of reporting analytical data and uses of statistics.

2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the concepts of mean, median and standard deviation
- Explain the different types of errors, their sources and their effects on analytical results
- Analyse methods of reporting analytical data

- Evaluate statistical evaluation of data-indeterminate errors
- Discuss uses statistics

NOTES

2.2 ANALYTICAL ERRORS AND THEIR EVALUATION

In analytical chemistry, the term 'error" has two slightly different meanings.

1. It refers to the difference between a measured value and the 'true' or 'known' value.
2. It often denotes the estimated uncertainty in a measurement or experiment.

Measurements invariably involve errors and uncertainties. It is impossible to perform a chemical analysis that is totally free of errors or uncertainties. We can only hope to minimize errors and estimate their size with acceptable accuracy. Errors are caused by faulty calibrations or standardizations or by random variations and uncertainties in results. Frequent calibrations, standardizations, and analyses of known samples can sometimes be used to lessen all but the random errors and uncertainties.

The term 'evaluation' can be defined as the process of determining the merit, worth, and significance of a subject using criteria that are defined by a set of standards.

2.2.1 Mean and Median

Usually, the mean or the median is used as the central value for a set of replicate measurements. An analysis of the variation in the data allows us to estimate the uncertainty associated with the central value. let us discuss them in detail.

Mean

The mean, also called the arithmetic mean or the average, is obtained by dividing the sum of replicate measurements by the number of measurements in the set. The symbol $\sum$xi means to add all of the values xi for the replicates; xi represents the individual values of '*x*' making up the set of N replicate measurements.

Median

It can be represented as,

$$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$$

- The median is the middle value in a set of data that has been arranged in numerical order.
- The median is used advantageously when a set of data contain an outlier. An outlier is a result that differs significantly from others in the set.
- An outlier can have a significant effect on the mean of the set but has no effect on the median.

Example: Results from six replicate determinations of iron in aqueous samples of a standard solution containing 20.0 ppm iron(III). Note that the results range from a low of 19.4, 19.5, 19.6, 19.8, 20.1 and 20.3 ppm of iron. Calculate the mean and median.

Solution:

$$\text{Mean} = \frac{19..4+19.5+19.6+19.8+20.1+20.3}{6} = 19.78$$

The average, or mean value, x, of the data is 19.78 ppm, which rounds to 19.8 ppm Fe.

Since the set contains an even number of measurements, the median is the average of the central pair. This can be calculated as,

$$Media = \frac{19.6 + 19.8}{2} = 19.7\ ppm\ Fe$$

2.2.2 Precision-Standard Deviation and Relative Standard Deviation

Precision describes the agreement among several results obtained in the same way. It describes the reproducibility of measurements.It is is readily determined by simply repeating the measurement on replicate samples. It is a set of replicate data may be expressed as standard deviation, variance, and coefficient of variation.

The standard deviation of a given set of data is defined as the square root of the sum of the individual deviations squared, divided by the number of readings.

$$S.D = \sigma = \sqrt{d12 + d22 + \ldots\ldots + dn2} / n = \sqrt{\sum d2 / n}$$

Relative standard deviation is the further measure of precision. The Relative Standard Deviation (RSD) is defined as the ratio of the standard deviation and mean of the set of data. RSD is expressed in ppt. RSD = s/ x X 1000 ppt.

There is a term 'variance' that also must be studied. It is defined as mean square deviation.

$$\text{i.e, Variance } V = \frac{\Sigma d2}{n}$$

2.2.3 Accuracy– Absolute and Relative Error

Accuracy indicates the closeness of the measurement to the true or accepted value and is expressed by the error. It measures agreement between a result and the accepted value. It is often more difficult to determine because the true value is usually unknown. An accepted value must be used instead. Accuracy is expressed in terms of either absolute or relative error.

The absolute error of a measurement is the difference between the measured value (x_i) and the true value (x_t). If the measurement result is low, the sign is negative; if the measurement result is high, the sign is positive. Example: Absolute error in the micro-Kjeldahl determination of nitrogen (Figure 2.1)

$$E_a = x_i - x_t$$

NOTES

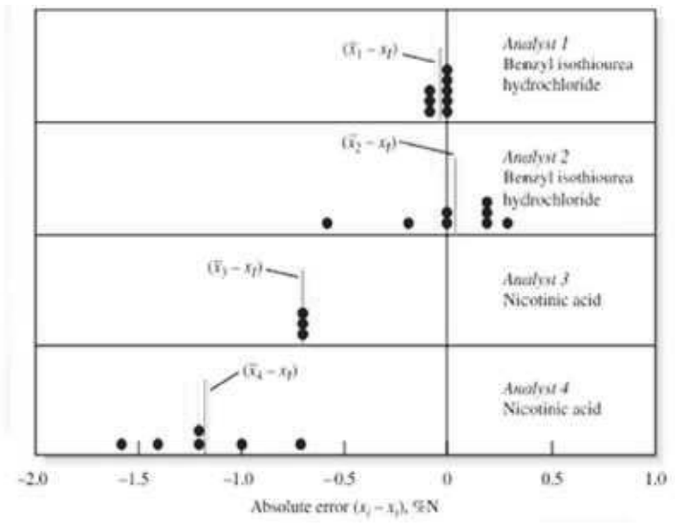


Fig. 2.1 *Absolute error*

The relative error of a measurement is the absolute error divided by the true value (x_t). Relative error may be expressed in percent, parts per thousand, or parts per million (ppm), depending on the magniture of the result.

$$E_r = \frac{E_a}{x_t} \times 100\%$$

2.2.4 Types of Errors in Experimental Data: Determinate Systematic, Indeterminate or random and Gross

Chemical analysis is affected by the following two types of errors:

- **Systematic (or determinate) error**: This error causes the mean of a data set to differ from the accepted value. For example, glassware and instrumentation.
- **Random (or indeterminate) error**: This error causes data to be scattered more or less symmetrically around a mean value. For example, environment and analyst
- **Gross error**: It is the third type of error which is different from both indeterminate and determinate errors. They usually occur only occasionally, are often large, and may cause a result to be either high or low. They are often the product of human errors. Gross errors lead to outliers, results that appear to differ markedly from all other data in a set of replicate measurements

Now let us study systematic error in detail.

Systematic errors have a definite value, an assignable cause, and the same magnitude for replicate measurements made in the same way. They lead to bias in measurement results. There are three types of systematic errors, which are as follows:

NOTES

a) Instrumental errors

Some of the common features of such errors are as follows:

- They are caused by nonideal instrument behavior, faulty calibrations, or by use under inappropriate conditions.
- Pipets, burets, and volumetric flasks may hold or deliver volumes slightly different from those indicated by their graduations.
- Calibration eliminates most systematic errors of this type.
- Electronic instruments can be influenced by noise, temperature, pH and are also subject to systematic errors.
- Errors of these types usually are detectable and correctable.

b) Method errors

Some of the common features of such errors are as follows:

- The nonideal chemical or physical behaviour of the reagents and reactions on which an analysis is based often introduce systematic method errors.
- Such sources of nonideality include the slowness of some reactions, the incompleteness of others, the instability of some species, the lack of specificity of most reagents, and the possible occurrence of side reactions that interfere with the measurement process.
- Errors inherent in a method are often difficult to detect and hence, these errors are usually the most difficult to identify and correct.

c) Personal errors

Some of the common features of such errors are as follows:

- They result from the carelessness, inattention, or personal limitations of the experimenter.
- Many measurements require personal judgments.
- Examples include estimating the position of a pointer between two scale divisions, the colour of a solution at the end point in a titration, or the level of a liquid with respect to a graduation in a pipet or buret.
- Judgments of this type are often subject to systematic, unidirectional errors.
- A universal source of personal error is prejudice, or bias.
- Number bias is another source of personal error that varies considerably from person to person.
- The most frequent number bias encountered in estimating the position of a needle on a scale involves a preference for the digits 0 and 5.
- Also common is a prejudice favoring small digits over large and even numbers over odd.
- Digital and computer displays on pH meters, laboratory balances, and other electronic instruments eliminate number bias because no judgment is involved in taking a reading.

NOTES

The errors which remain even after the systematic errors have been taken care of is known as residual or random errors. They arise due to the large number of small factors that change from one measurement to another. The changes may be sometimes such that we are not aware. These changes or disturbances are lumped together and are called random or residual.

2.2.5 Sources of Errors and the Effects Upon the Analytical Results

The common sources of measurement errors are as follows:

(i) Human error: It is also known as gross error. It can be prevented by careful planning and execution of the experiment.

(ii) Reading error: It is a combination of the instrument's accuracy and precision and can be found in the manufacturer's specifications for the instrument. It is treated under the general categories of systematic error and random error. The ability of an instrument to measure the true value of a characteristic is referred to as accuracy. This indicates how near the measurement is to the actual value. The randomness of the measured value owing to fluctuation in the measuring apparatus is referred to as precision. This refers to the measurement's repeatability.

(iii) Systematic error: It is a determinate error. It has the same sign and magnitude for identical conditions. It can often can be removed or compensation made. It must be rectified before reporting data or used in subsequent calculations.

Such errors are predictable. Its sources are as follows:

- **Mis-calibration of instruments:** This class of systematic error refers to the instrument's accuracy. Could be due to a zero offset or improper instrument span.
- **Natural phenomena or inherent characteristics of the instrument**: Could be due to hysteresis or the linearization of a non-linear response, or could be due to the method used, i.e. measuring surface temperature of a pipe to represent fluid temperature.

(iv) Random error: This is a combination of the randomness of the measurement process and the randomness of the characteristic you are measuring. It is also called indeterminate error. They can be positive or negative and have varying magnitude. These errors are not predictable. The can be quantified using statistical methods.

Sources of random error are random process fluctuations, i.e., equipment 'goblins', moon phase, miscellaneous, random instrument fluctuations and degree of subdivision of instrument scale and ability to precisely read the scale.

Check Your Progress

1. What are random errors?
2. How do you calculate mean?
3. Mention the types of systematic errors.

2.3 METHODS OF REPORTING ANALYTICAL DATA

NOTES

Reporting an analytical data can be done in many ways. Some of the rules for reporting analytical data are as follows:

Rule 1. In expressing an experimental data never retain more than one doubtful digit. Eliminate all digits that are not significant.

Rule 2. Retain as many significant figures in a result or in any data as will give only are uncertain figure. For example, a volume between 25.5 cm^3 and 25.7 cm^3 should be written as 25.6 cm^3, and not as 25.60 cm^3, since the latter would indicate that the value lies between 25.59 and 25.61 cm^3.

Rule 3 (a). In rounding off quantities to the correct number of significant figures, add one to the last figure retained if the following figure is 5 or over. This is known as rounding up. Thus the number 8.856 has been rounded upto the digit 8.86.

(b) If the last figure discarded is less than 5, leave the last digit of the number unchanged. This is called rounding down. For example, the numb 7.64 is rounded to 7.6. It must be noted that rounding never changes the power of 10. Therefore it is better to express numbers in exponential notation before rounding. For instance, in rounding 67832 to four figures, the value is 6.783×10^4.

Rule 4. In addition or subtraction in each number, , there should be only as many significant figures as there are in the least accurately known number. Thus the addition of the three numbers, 168.11, 7.045 and 0.6832 should be written as 168.11 + 7.05 + 0.68 = 175.84 since there are only two significant numbers in the least accurately known number. The sum on difference of two or more quantities cannot be more precise than the quantity having the largest uncertainty.

Rule 5. In multiplication or division, retain in each factor one more significant figure than is contained in the factor having the largest uncertainty. The percentage precision of a product or quotient cannot be greater than the percentage precision of the past precise factor entering into the calculation. Thus, the multiplication of four numbers, 1.26, 1.336, 0.5834 and 25.8652 should be done using the values $1.26 \times 1.336 \times 0.583 \times 25.87$ and the result expressed to three significant figures.

Rule 6. Computation involving a precision not greater than one fourth of 1% should be made with a 10-inch slide rule. Slide rule is a good method for checking the calculations made by logarithms. Use of logarithms has been recommended when a large number of multiplications and divisions are to be made.

2.3.1 Statistical Evaluation of Data-Indeterminate Errors

Indeterminate errors are the outcome of uncertainties in a measurement that are unknown and which cannot be controlled by the experimentalist. They appear rarely and not get eliminated. A synthetic standard material's overall composition must closely resemble the composition of the samples to be examined. It is critical to guarantee that the analyte concentration is precisely understood. Unexpected interferences may not be shown by a synthetic standard, leaving the accuracy of determinations unknown.

NOTES

An error is the difference between a single result estimate of a quantity and its true value. This difference or deviation (positive or negative) may be expressed as follows:

- In the units in which the quantity is measured
- As a percentage of the true value

Many kinds of error may skew the findings of laboratory tests. These include random error, random variation, constant and proportional determinate (systematic) error, and methodological or physiologic interference. A brief decription of these types of errors is given here:

Random errors

Random errors occur haphazardly and unpredictably. It may occur by:

- Using wrong patient's specimen
- Incorrectly reconstituting reagent

Other types of random errors include:

- Misreading of instruments
- Chance errors in calculation
- Transcription error, including transposing of digits

Random errors cause imprecision; these errors are inherent in all analyses. Sources of random variation influence each measurement differently; the result may be higher or lower than it should be and may vary considerably or little in magnitude.

Determinate error

Determinate error is also called systematic error. It can be traced to a specific cause. Common types these errors include:

Constant systematic errors: Constant systematic error is an error whose direction and magnitude does not change even when the concentration of the analyte changes.

Proportional systematic error: Proportional systematic error is an error that always occurs in one direction.

A classic example of systematic error is a method-related error caused by the presence of interfering substances in the specimen. These interfering substances may be endogenous metabolites, such as bilirubin, chylomicrons, drugs received by the patient or an excess of anticoagulant.

Drug interference may be methodological (in vitro) or physiological (in vivo) in origin. Methodological interference occurs when the drug is actually detected during measurement as if it were the substance of interest. Physiologic interference, which actually represents biological variation occurs when a drug received by the patient induces an increase in the substance of interest being measured.

Analytical errors

These errors are classified into random analytical errors and systematic analytical errors.

NOTES

Random analytical errors: These errors indicate poor precision. Random analytical errors include pipetting error, transcription error, wrong sample numbering and labeling and errors in instrumentation.

Systematic errors: These errors indicate poor accuracy. Systematic analytical errors could occur due to wrong procedure, incorrect standard and incorrect standardization procedure. In order to set up a statistical quality control programme the first step is to determine standard deviation (S) for the procedure.

2.3.2 Uses of Statistics

Some of the common uses of statistics are as follows:

- Statistics aids in the better comprehension and accurate description of natural events. In every subject of study, statistics aids in the effective and efficient design of a statistical enquiry.
- Statistics aids in the collection of useful quantitative data.
- Statistics aids in the presentation of complex data in a tabular, diagrammatic, or graphic format that is easy to understand.
- Through quantitative observations, statistics aids in understanding the nature and pattern of variability of a phenomenon.
- Statistics aids in the development of valid inferences about population parameters from sample data, as well as a measure of their reliability.

Check Your Progress

4. Write the rule in multiplication or division while reporting analytical data.
5. What is the outcome of unknown and uncontrollable uncertainties in a measurement?

2.4 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Random (or indeterminate) error, causes data to be scattered more or less symmetrically around a mean value.
2. The mean, also called the arithmetic mean or the average, is obtained by dividing the sum of replicate measurements by the number of measurements in the set.
3. The three types of systematic errors are as follows:

   i. Instrumental errors

   ii. Method errors

   iii. Personal errors
4. In multiplication or division, retain in each factor one more significant figure than is contained in the factor having the largest uncertainty.
5. Indeterminate errors are the outcome of uncertainties in a measurement that are unknown and which cannot be controlled by the experimentalist.

NOTES

2.5 SUMMARY

- The term 'error' refers to the difference between a measured value and the 'true' or 'known' value.
- The term 'evaluation' can be defined as the process of determining the merit, worth, and significance of a subject using criteria that are defined by a set of standards.
- Measurements invariably involve errors and uncertainties.
- The mean or the median is used as the central value for a set of replicate measurements.
- The median is the middle value in a set of data that has been arranged in numerical order.
- The standard deviation of a given set of data is defined as the square root of the sum of the individual deviations squared, divided by the number of readings.
- The RSD is defined as the ratio of the standard deviation and mean of the set of data.
- Precision describes the agreement among several results obtained in the same way.
- Accuracy indicates the closeness of the measurement to the true or accepted value and is expressed by the error.
- The absolute value is the difference between the measured value and the true value.
- The relative error of a measurement is the absolute error divide by the true value.
- Chemical analysis is affected by systematic and random errors.
- The common sources of measurement errors are human error, reading error, systematic error and random error.
- Systematic errors may be either constant or proportional.
- The magnitude of a constant error stays essentially the same as the size of the quantity measured is varied.
- Proportional errors decrease or increase in proportion to the size of the sample.
- Statistics has several uses, such as better comprehension, efficient designing, collection of useful quantitative and data development of valid inferences.

2.6 KEY TERMS

- **Absolute Error:** This is the difference between the measured value and the true value.
- **Relative Error:** This is the absolute error divide by the true value.
- **Evaluation:** This term refers to the act or outcome of evaluating a situation that requires careful evaluation.

- **Accuracy:** This term refers to the closeness of the measurement to the true or accepted value and is expressed by the error.
- **Precision:** This term refers to the agreement among several results obtained in the same way.

NOTES

2.7 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Differentiate between constant and proportional errors.
2. How is accuracy different than precision?
3. What are the uses of statistics?
4. Mention the features of instrumental errors.

Long-Answer Questions

1. Discuss the different sources of errors and their effects on analytical data.
2. Explain how statistical evaluation of data-indeterminate errors is performed.
3. Analyse the methods of reporting analytical data.

2.8 FURTHER READING

Dash, Dhruba Charan. 2011. *Analytical Chemistry*. New Delhi: Prentice Hall India Pvt, Limited

Hibbert, D.B. and J.J. Gooding. 2006. *Data Analysis for Chemistry*. UK: Oxford University Press.

Benwart, G. J. 1987. *Basic Food Microbiology*. New Delhi: CBS Publishers & Distributors.

Christian, Gary D. 2007. *Analytical Chemistry*, 6th Edition. Noida: Wiley India Pvt. Limited

UNIT 3 FOOD ANALYSIS

Structure

NOTES

3.0 INTRODUCTION

Food may be contaminated from outside sources on the way from the field to the processing plant, or during transport, storage and distribution. There are thousands of different types of micro-organisms everywhere in air, water, soil and foods, and in the digestive tract of animals and human. Fortunately, the majority of microbes usefully functions in the environment and in some branches of food industry, such as production of wine, beer, dairy products, bakery products, etc. On the other hand unwanted food spoilage is generally caused by microbes and contamination of food with pathogens causes food safety problems. The microorganisms occurring on and in foods are divided into three groups: bacteria, yeast and moulds. This unit will discuss concept of food-adulteration and common adulterants and their microscopic adulterants in food. It will also discuss pesticide analysis in food products along with the different methods of extraction and purification of sample. In addition, it will introduce the concept of gas chromatography and thin-layer chromatography for identification of organophosphates and chlorinated pesticides, respectively in food products.

3.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the concept of food adulteration
- Analyse common adulterants and contamination of food
- Discuss pesticide analysis in food products
- Explain methods of extraction and purification of sample

NOTES

3.2 ANALYSIS OF FOOD: CARBOHYDRATES; CRODE PROTEIN, VITAMINS, MOISTURE, ASH, CRUDE FIBRE, CALCIUM, POTASSIUM, SODIUM AND PHOSPHATE

Food analysis is the study of analytical processes for describing the qualities of foods and their ingredients, as well as their creation, application. The types and proportions of nutrients present in the food are all important in determining what organisms are most likely to grow. Consideration must be given to: (i) foods for energy (ii) goods for growth (iii) accessory food substances, or vitamins, which may be necessary for energy or growth. Let us study various components in detail.

- **Carbohydrates:** One of the main energy rich components in food is carbohydrates. Microorganisms depend on these for their energy requirements. They break down the sugar present in the food into simpler sugars for their consumption. Very few microorganisms are able to utilize complex carbohydrates such as starch and cellulose as sources of energy by first degrading these compounds into simple sugars. Fats are also used by microorganisms as source of energy, but these compounds are attacked by a relatively small number of microbes in foods. Carbohydrates, especially the sugars, are most commonly used, but other carbon compounds may also serve as carbon source, for example esters, alcohols, peptides, amino acids, and organic acids and their salts. Complex carbohydrates, for example cellulose, can be utilized by comparatively few organisms, and starch can be hydrolysed by only a limited number of organisms. Microorganisms differ even in their ability to use some of the simpler soluble sugars. Many organisms cannot use the disaccharide lactose (milk sugar) and therefore do not grow well in milk. Maltose is not attacked by some yeasts. Bacteria often are identified and classified on the basis of their ability or inability to utilize various sugars and alcohols. Most organisms, if they utilize sugars at all can use glucose.
- **Fats:** A limited number of types of microorganisms can obtain their energy from fats but do so only if a more readily usable energy food, such as sugar, is absent. First, the fat must be hydrolysed with the aid of lipase to glycerol and fatty acids, which then can serve as an energy source for the hydrolyzing organism or other organisms. In general, aerobic microorganisms are more commonly involved in the decomposition of fats than are anaerobic ones, and the lipolytic organisms usually are also proteolytic. Direct oxidation of fats containing unsaturated fatty acids usually is chemical.
- **Proteins:** Hydrolysis products of proteins, i.e., peptides and amino acids, serve as energy source for many proteolytic organisms when a better energy source is lacking and as foods for energy for other organisms that are not proteolytic. Meats, for example, may be low in carbohydrate and therefore decomposed by proteolylic species, for example Pseudomonas species, with successive growth of weakly proteolytic or non-proteolytic species that can utilize the products of protein hydrolysis. Organisms differ in their

NOTES

ability to use individual amino acids for energy. Microorganisms differ in their ability to use various nitrogenous compounds as the source of nitrogen for growth. Many organisms are unable to hydrolyse proteins and, hence, cannot get nitrogen from them without the help from a proteolylic organism. One protein may be a better source of nitrogenous food than another because of different products formed during hydrolysis, especially peptides and amino acids. Peptides, amino acids, urea, ammonia, and other simpler nitrogenous compounds may be available to some organisms but not to others or may be usable under some environmental conditions but not under others. Some of the lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein, and do not grow well with only a limited number of types of amino acids present. The presence of fermentable carbohydrates in a substrate usually results in acid fermentation and suppression of proteolytic bacteria and, hence, in what is called a 'sparing' action on the nitrogen compounds. Also, the production of obnoxious nitrogenous products is prevented or inhibited. Many types of moulds are proteolytic, but comparatively few genera and species of bacteria and very few yeasts are actively proteolytic.

In general, proteolytic bacteria grow best at pH values near neutral and are inhibited by acidity, although there are exceptions, such as proteolysis by the acid-proteolytic bacteria that hydrolyse protein while producing acid. The basic source of nitrogen for the microorganisms is the protein. A large number of various microorganisms breaks down and thrive on the amino acids present in the protein rich foods stuffs. Some microbes, for example, are able to utilize nucleotides and free amino acids, while others are able to utilize peptides and proteins.

- **Accessory food substances or vitamins:** Some microorganisms are unable to manufacture some or all of the vitamins needed and must have them furnished. Most natural plant and animal food-stuffs contain an array of these vitamins, but some may be low in amount or lacking. Thus, meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid. Egg white contains biotin but also contains avidin, which ties up biotin, making it unavailable to microorganisms and eliminating as possible those spoilage organisms which must have biotin supplied. The processing of foods often reduces the vitamin content. Thiamine, pantothenic acid, the folic acid group, and ascorbic acid (in air) are heat labile, and drying causes the loss of vitamins such as thiamine and ascorbic acid. Even storage of foods for long periods—especially if the storage temperature is elevated— may result in a decrease in the level of some of the accessory growth factors.
- **Moisture:** Moisture content affects shelf life because more water in a product makes it more susceptible to germs, which can cause the food to rot and be damaged. As a result, maintaining a fixed level of moisture in a product allows producers to precisely anticipate a product's shelf life, ensuring that consumers are not exposed to damaged food.
- **Ash:** Any inorganic particle detected in food is referred to as ash. The residual substance is referred to as ash after all the water content and organic

NOTES

elements have been removed from the food. Food scientists frequently burn items to this point in order to investigate the ash left behind and gain a better understanding of the contents.

- **Crude fibre:** It aids in restoring the gastrointestinal tract's natural capacity and makes it easier to eliminate the remnants of food digesting. The high fibre content increased the laxative effect. The higher the readiness of fibres in meals to absorb water, the stronger the laxative effect.
- **Calcium:** It is required for practically every bodily function. It is not produced by the body. Calcium can be obtained through foods and supplements, but its absorption takes place by the body better than food. Microorganisms' calcium requirements had assumed specificity for chelation agents like EGTA and A23187, which the reagents lacked.
- **Potassium:** It is involved in each and every heartbeat. It helps your heart squeeze blood through your body a hundred thousand times per day. It also aids in the movement of your muscles, the functioning of your neurons, and the filtering of blood by your kidneys. Eating fruits and vegetables is the greatest method to obtain adequate potassium.
- **Sodium:** It is a mineral that the body requires to function properly. Sodium is found in salt. Sodium is used by the body to regulate blood pressure and blood volume. Your muscles and nerves also require sodium to function properly. Sodium is found in almost all foods. Sodium chloride, also known as table salt, is the most prevalent type of sodium.
- **Phosphate:** Many foods naturally contain phosphorus, however food processing can also increase phosphorus levels. Phosphorus is used by the body to keep bones strong and healthy. Phosphorus also assists in the removal of waste and the restoration of damaged tissues. The majority of people obtain adequate phosphorous from their food.

Check Your Progress

1. Define food analysis.
2. What does the term 'ash' referred to in food science?
3. Which mineral is used in the removal of waste and the restoration of damaged tissues??

3.3 FOOD ADULTERATION

Any article substance with nutritional value consumed by humans for there is growth is called food

- Any article which ordinarily enters into or is used in the composition or preparation of human food.
- Any flavouring matter or condiments.
- Any other article which the Central Government may having regard to its use, nature, substance or quality, declare, by notification in the official gazette as food for the purpose of this Act.

Let us study about food and its adulteration.

Adulteration is a process that includes adding some materials or elements, especially inferior one to make a food item impure or alter its original form. Any material which is or could be employed for the purposes of adulteration is known as adulterant. An article of food shall be deemed to be adulterated:

- If the article sold by vendor is not of the nature, substance or quality demanded by the purchaser.
- If the article contains any other substance which affects the substance or quality thereof.If any inferior or cheaper substance has been substituted wholly or in part for the article so as to affect the nature, substance or quality of the product.
- If any constituent of the article has been wholly or in part extracted to affect the quality thereof.
- If the article has been prepared, packed or kept under unsanitary conditions whereby it has become contaminated or injurious to health.
- If the article consists wholly or in part of any filthy, putrefied, rotten decomposed or diseased animal or vegetable substance or is insect-infested or is otherwise unfit for human consumption.
- If the article is obtained from a diseased animal.
- If the article contains any poisonous or other ingredient which renders it injurious to health.
- If the container of the article is composed, whether, wholly or in part of any poisonous or deleterious substance which renders sits contents injurious to health.
- If any colouring matter other than that prescribed in respect thereof is present in the article or if the amounts of the prescribed colouring matter which is present in the article are not within the prescribed limits.
- If the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits.
- If the quality or purity of the article falls below the prescribed limits of variability which renders it injurious to health.
- If the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities not within the prescribed limits of variability which renders it injurious to health.

Procedure for sampling and analysis

Any food inspector can enter and inspect any place where any article of food is manufactured or stored for sale or stored for the manufacture of any other article of food for sale or exposed or exhibited for sale or where any adulterant is manufactured or kept and take samples of such article of food or adulterant for analysis.

For sampling and analysis, follows the given steps:

1. Notice will be issued by the inspector in writing then and there to the seller indicating his intention.

NOTES

2. Three samples are taken and the signature of the seller is affixed to them.
3. One sample is sent for analysis to public analyst under intimation to the local health authority.
4. The other two samples are sent to the local health authority for further reference.

Guilty will be punished with imprisonment for a term which shall not be less than six months and up to 3 years and with fine up to one thousand rupees.

3.3.1 Common Adulterants in Food

There are various adulterants in food, which are as follows:

- **Milk:** Water, chalk, urea, caustic soda, and skimmed milk are some of the adulterants used in milk, whereas paper, refined oil, and skimmed milk powder are used in Khoya.
- **Tea and coffee:** The two most consumed beverages in India are tea and coffee. Therefore, they are highly adulterated. Tea leaves are frequently contaminated by similar-colored leaves, some of which may or may not be edible. Several incidences of liver infection have been recorded around the country as a result of ingesting contaminated tea. On the other hand, coffee seeds are contaminated with tamarind seeds, mustard seeds, and chicori.
- **Wheat and other food grains:** It is very commonly adulterated with ergot, a fungus containing poisonous substances, which is extremely injurious to health.
- **Vegetables:** Various dyes and chemicals are used to colour various coloured and textured vegetables. Malachite green, a chemical colour that has been linked to cancer, is commonly found in these crops. Oxytocin sachharin, wax, calcium carbide, and copper sulphate are common adulterants in fruits and vegetables.
- **Sweets:** The most common ingredients in making these sweets are khoya and chenna and they're often adulterated with starch.
- **Honey:** There are different amounts of antibiotics and their consumption over time may cause resistance to antibiotics, lead to blood-related disorders and injury to the liver.
- **Dal:** Arhar dal is the most widely contaminated dal, and it is usually tainted with metanil yellow. Metanil yellow is a common non-permitted food colour used widely in India. Long-term ingestion of metanil yellow produces neurotoxicity in the developing and adult brain.
- **Spices:** Adulterants in asafoetida include soap stone or other earthy materials, as well as foreign resin. Because they are practically identical in size yet tasteless, papaya seeds and black berries are commonly employed as adulterants in black pepper (sometimes bitter). Brick powder, salt powder, or talc powder, as well as artificial colours like Sudan Red, are used to adulterate red chilli powder. The world's most costly spice, saffron is adulterated by coloured dried tendrils of maize cob.

NOTES

- **Butter and cream:** Plant oils such as palm oil, sunflower oil, and soybean oil can be partially substituted for butter or diluted with water.
- **Ice cream:** Pepperonil, ethylacetate, butraldehyde, emil acetate, nitrate, washing powder, and other frequent adulterants in ice cream are all poisonous. Ethyl acetate causes terrible problems to the lungs, kidneys, and heart, and pepperoil is used as a pesticide.

3.3.2 Contamination of Food Stuffs

Following are the various types of contaminations that can be seen on the various food items:

(i) Spoilage of fruits and vegetable juices

It has been estimated that one-fourth of all fruit produce harvested is spoiled before consumption. Spoilage of fresh fruits and vegetables usually occurs during storage and transport and while waiting to be processed. Unlike many other foods,fruits and vegetables after picking and before processing are 'alive' for an extended time. The resulting respiration of these products and the normal ripening process complicate an independent discussion of the microbiological spoilage of fruits and vegetables.

Fig. 3.1 *Fruits Showing Microbial Growth*

Contamination of fruits and vegetables takes place in the following stages:

1. **During Transportation:** As soon as fruits and vegetables are gathered into boxes, lugs, baskets, or trucks during harvesting, they are subject to contamination with spoilage organisms from each other and from the containers unless these have been adequately sanitized. During transportation to market or the processing plant, mechanical damage may increase the susceptibility to decay and growth of microorganisms and refrigeration during transportation will slow such growth. Washing the fruit or vegetable may involve a preliminary soaking or may be achieved by agitation in water, or preferably, by a spray treatment. Soaking and washing by agitation tend to distribute spoilage organisms from damaged to whole foods. Reused water is likely to add organisms, and the washing process may moisten surfaces enough to permit growth of organisms during a holding period. Washing with detergent or germicidal solutions will reduce the numbers of microorganisms on the foods.

NOTES

2. **Contamination in handling:** Sorting spoiled fruits or vegetables or trimming spoiled parts removes microorganisms, but additional handling may result in mechanical damage and therefore, greater susceptibility to decay. When these products are sold in the retail market without processing, they are not ordinarily subjected to much further contamination, except for storage in the market in contaminated bins or other containers, possible contact with decaying products, handling by salespeople and customers, and perhaps spraying with water or packing with chipped ice. This spraying not only gives a fresh appearance to the vegetables and delays decomposition, but also adds organisms, for example, psychrotrophs, from water or ice and gives a moist surface to encourage their growth on longer storage.
3. **Contamination in processing:** In the processing plant, fruits and vegetables are subjected to further contamination and chances for growth of microorganisms, or numbers and kinds of organisms may be reduced by some procedures. Adequate washing at the plant causes a reduction in numbers of microorganisms on the food, as do peeling by steam, hot water, or lye and blanching (heating to inactivate enzymes, etc.). Sweating of products during handling increases numbers, processes such as trimming, mechanical abrasion or peeling, cutting, pitting or coring, and various methods of disintegration may add contaminants from the equipment involved. In fact, every piece of equipment coming in contact with the food can be a significant source of microorganisms unless it has been cleaned and sanitized adequately. Modern metal equipment with smooth surfaces and without cracks, dead ends, etc., is made to facilitate such treatments. Examples of possible sources of contamination of foods with microorganisms are trays, bins, tanks, pipes, flumes, tables, conveyor belts and aprons, fillers, blanchers, presses, screens, and filters. Wooden surfaces are difficult to clean and sanitize and therefore are especially likely to be sources of contamination, as are cloth surfaces, for example, on conveyor belts. Neglected parts of any food-handling system can build up numbers of microorganisms contaminating the food. Hot-water blanching, although it reduces the total numbers of organisms on the food, may cause the build up of spores of thermophilic bacteria, causing the spoilage of canned foods, for example, flat sour spores in peas.

Some of the other factors responsible for spoilage are as follows:

- **Contamination by microbes:** Build-up of populations of microorganisms on equipment as a result of microbial growth in the exudates and residues from fruits and vegetables may greatly influence the amount of contamination of the foods and the growth of the contaminants. Not only is there the possibility of the addition of large numbers of organisms from this source, there is also the likelihood that these organisms are in their logarithmic phase of growth and therefore able to continue rapid growth. This effect is especially evident on vegetables following blanching. The heat treatment reduces the bacterial content considerably, damages many of the surviving cells, and consequently lengthens their lag period. On the other hand, the actively growing contaminants from the equipment can attain large numbers if enough

time is allowed before freezing, drying, or canning; such growth is usually the cause of very high bacterial counts.

- **Contamination by spoiled items:** Inclusion of decayed parts of fruits increases the numbers of microorganisms in fruit juices. Numbers in orange juice, for example, and numbers of coliforms are increased greatly by the inclusion of fruits with soft rots. Heating grapes before extraction reduces numbers of organisms in the expressed juice, but pressing introduces contamination.
- **Contamination by equipment:** The kinds of microorganisms from equipment will depend on the product being processed, for that product will constitute the culture medium for the organisms. Thus, pea residues would encourage bacteria that grow well in a pea medium and in tomatoes those organisms which can develop in tomato juice. As the equipment is used throughout the day, the organisms can continue to build up. At the end of the run, however, when the equipment is cleaned and sanitized, the total numbers of microorganisms thereon are greatly reduced, and if the operation is efficient, only the resistant forms survive. Therefore, spores of bacteria are likely to survive, and if conditions for growth are present while the equipment is idle, these spore formers may increase in numbers, especially in poorly cleaned parts. The thermophilic spore formers so troublesome to canners of vegetables build up in this manner and add to the difficulty of giving the foods an adequate heat process. The numbers of such organisms on poorly cleaned and sanitized equipment may be high at the start of a day's run and decrease as the day progresses, but the reverse usually is true. A layoff during the run permits a renewed increase in numbers. It is obvious that the numbers of microorganisms that enter foods from equipment depend on the opportunities given to these organisms for growth and that these opportunities are the result of inadequacy of cleaning and sanitizing, combined with favourable conditions of moisture and temperature for an appreciable period of time.

(ii) Contamination and spoilage of cereals

Harvested grains are infected by microorganisms from soil and insects. Grains are infected by bacteria which belong to *Pseudomonadaceae* and *Lactobacillaceae*. Washing the grains remove some of the microorganisms. The milling processes further reduce the number of organisms. Bacteria in wheat flour contain spores of Bacillus. A freshly baked loaf of bread is practically free of viable microorganisms, but is subject to contamination by mold spores from air during cooling and before wrapping. During slicing, contamination takes place from microorganisms in the air. The contamination of grains and cereal products with moulds is a major concern because of the presence of mycotoxins. To reduce contamination by moulds and to avoid conditions which allow their growth is a major challenge for microbiologists undertaking research activity in the field.

Cereals are important foods which provide bulk of our dietary requirements. They are also source of carbohydrates which are metabolized by body for energy generation. Besides, cereals also provide minerals, proteins and vitamins.

NOTES

Types of cereal products

The types of cereals products are as follows:

- **Whole cereals:** Only the husk of the grain is removed, for example rice, wheat, gram, lentils, etc.
- **Milled grain products:** They are made by removing the bran and usually the germ of the seed and then crushing the kernel into various sized pieces, for example wheat flour, maida, semolina (rawa), etc.
- **Processed cereals:** They incluse weaning food, breakfast cereals, breads, etc.
- **Ready ixes:** They incluse cake and bakery products, pasta, macaroni.

Various spoilage factors influencing the quality of cereals are as follows:

- **Physical:** Physical losses are caused by spillages, which occur due to use of faulty packaging materials.
- **Physiological:** Physiological losses include respiration and heating in grains, temperature, humidity and oxygen.
- **Biological:** Biological losses occur due to micro-organisms, insects, rodents, natural microflora of harvested grains.

Cereal grains and meals

Due to low moisture content grains and flours usually have long shelf life if these are properly harvested or stored under proper conditions where microbial growth is not supported. Moulds can rapidly grow on grains and corn when these products are stored in at high temperature and humidity. For example, ear rot of corn (Refer Figure 3.2) can result in major economic losses. The spoilage mainly occurs due to moisture absorption during storage leading to fungal growth. Wet mash of the grains or a mash of the meals will undergo an acid fermentation by lactic acid bacteria and coliform normally present on plant surfaces. This is followed by alcoholic fermentation by yeasts as soon as the acidity has increased enough to favor them. Finally moulds will grow on the top surfaces, where air is available. Before bulk packaging and storage, the whole grains are fumigated to reduce microbial load and increase storage period.

Fig. 3.2 *Fungal Spoilage of Corn*

Major factors involved in spoilage of stored grains by moulds are:

- Type and number of microorganisms.
- Moisture content of more than 12-13%.
- Storage temperature.
- Physical damage.

Common bacterial genera spoiling grains are *Pseudomonas*, *Micrococci*, *Lactobacillus* and *Bacillus*. Common moulds spoiling grains are as follows:

- *Aspergillus, Penicillium, Rhizopus, Mucor, Fusarium.*
- Many of these moulds can produces mycotoxins. For example: Stem rot and head blight of wheat (Refer Figure 3.3) and barley caused by *Fusarium culmorum* and *Fusarium graminearum.*

Fig. 3.3 *Head Blight of Wheat*

Contamination of Grains by the Ascomycete: *Claviceps purpura* causes ergotism, a toxic condition. Hallucinogenic alkaloids produced by this fungus can lead to altered behavior, abortion, and death if infected grains are eaten.

(a) Spoilage of Flours

The process of flour making such as washing and the milling reduce the microbial content and hence microbial load. Moisture content of less than 15% does not allow growth of moulds. Most moulds and bacteria in flours can grow only above 17% moisture, thus moistening of flours is essential for spoilage by microbes. Because of the variations in microbial content of different lots of flour, different type of spoilage result. If acid-forming bacteria are present, an acid fermentation begins, followed by alcoholic fermentation by yeasts and then acetic acid by *Acetobacter species*.

In the absence of lactis and coliforms, micrococci have been found to acidify the paste, and in their absence, species of *Bacillus* produce lactic acid, gas, alcohol, acetone, small amount of esters and other aromatic compounds, which develop characteristic odor.

(b) Spoilage of bread

Bread is a major product prepared using flours. Dough is prepared from flours which undergo fermentation for which desirable microorganisms must grow. If this fermentation exceeds the required limits, it causes souring. Excessive growth of

NOTES

proteolytic bacteria reduces the gas holding capacity which is otherwise required for dough rising (Refer Figure 3.4).

Bacterial spoilage of bread

Following are the bacterial spoilage of bread:

- The chief causative organism is mucoid variant of *Bacillus subtilis or B. licheniformis,* with their spores surviving baking temperatures (not more than 100C). These spores can germinate once they get favourable conditions.
- The ropy condition is the result of capsulation of the bacillus, together with hydrolysis of the flour proteins (gluten) by proteinases of the organism and of starch by amyla to give sugars that encourage rope formation.
- In one stage the slimy material can be drawn out into long threads when the bread is broken and pulled apart.
- This spoilage is characterized by development of white chalk like spots, which are caused by growth of yeast like fungi *Endomycopsis fibuligera* and *Trichonosporon variable.*
- Due to the growth of bacteria *Serratia marcescens* which produces brilliant red color on starchy foods giving blood like appearance.

Mould spoilage of bread

Following are the mould spoilage of bread:

- **Green spored mould:** The green growth most likely is *Penicillium expansum* or *P. stoloniferum; A. niger* with its greenish or purplish brown to black conidial heads and yellow pigment diffusing into the bread.
- **Black mould:** The black growth is characteristic of *Rhizopus stolonifer Rhizopus stolonifer with* its white cottony mycelium and black dots of sporangia.
- **Red bread mould:** It includes the following:
  - o *Monilia (Neurospora) sitophila,* whose pink conidia give a pink or reddish color to its growth.
  - o A red color in the crumb of dark bread has been caused by *Geotrichum aurantiacum.*

Fig. 3.4 *Fungal Spoilage of Bread*

The factors responsible for mold spoilage are as follows:

- Heavy contamination after baking, due to air heavily laden with mold spores, a long cooling time, considerable air circulation, or a contaminated slicing machine.
- Slicing in that more air is introduced in to the leaf.
- Wraping, especially when bread is still warm.
- Storage in a warm humid place.

(iii) Contamination and spoilage of fish

Fish is a very perishable food due to its high nutritional content. Fish have a high content of non-protein nitrogen, and autolytic changes caused by their enzymes increase the supply of nitrogenous foods (amino acids and amines) and glucose for bacterial growth, which produce trimethylamine, ammonia, lower fatty acids, aldehydes, hydrogen sulfides, mercaptans, indoles which are indicative of putrefaction.

Storage and processing conditions also affect microbial growth, which are as follows:

- **Chilling temperature:** *Pseudomonas*, *Acinetobacter, Moraxella* and *Flavobacterium*.
- **Higher temperature:** *Micrococcus, Bacillus*.
- Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria an*d Photobacterium phosphoreum*.
- Heavily wet-salted fish support growth of yeasts.
- Salt fish are spoiled halotolerant or by halophilic bacteria of the *genera Serratia, Micrococcus, Bacillus, Alcaligenes, Pseudomonas.*
- Smoked fish are mainly spoiled by moulds.
- Addition of organic acid select for lactic acid bacteria and yeasts.
- A musty or muddy odor and taste of fish has been attributed to the growth of *Streptomyces* species in the mud at the bottom of the body of water and the absorption of the flavor by the fish.
- Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish.

The siscolouration of fish flesh. Occurs during spoilage. It can be seen as follows:

- Yellow to greenish yellow colors caused by Pseudomonas fluorescence, yellow micrococci
- Red or pink colors from growth of *Sarcina, Micrococcus, Bacillus s*p. or by moulds or yeasts
- Chocolate brown color by *Asporogenous* yeast

(iv) Contamination and spoilage of seafoods

The term seafood covers fish, shellfish, crabs, shrimps, oysters, clams, mollusks from all waters–fresh, marine, warm, or cold. Most shrimp, crabs, crawfish,

NOTES

lobsters, and other crustaceans are consumed fully cooked, and hence most bacteria, viruses, and parasites are either inactivated or reduced to a level at which infection may not occur. However, foods that are transported and consumed in an uncooked state are an increasingly important source of food-borne infection. The problem becomes more serious because of rapid movement of people and products around the world. International trade in uncooked foods, aided by rapid air transport, provides many opportunities for disease transmission.

Many molluscan shellfish (primarily oysters, clams, and mussels) are customarily consumed raw or with minimal heating and have caused foodborne illness outbreaks and mortality. Raw sewage can contaminate shellfish-growing areas; in addition, waterborne pathogens such as Vibrioare more prevalent in the water column during the warm months (for example, in Chesapeake Bay on the mid-Atlantic coast of the United States). Viruses also can be a problem. Oysters are filter feeders that process several liters of water per day, leading to the potential concentration of at least 100 types of enteric viruses. Heavy rainfall in shellfish areas can cause runoff of pathogens from adjacent septic systems and contaminate coastal waters. Often it is necessary to ban shellfish harvesting until the animals void pathogens from their digestive systems. Alternatively, shellfish from contaminated areas can be moved to clean waters to allow them to clean their digestive systems.

Table 3.1 *Organism Spoiling Seafoods*

Type of Seafood	**Spoiling Organism**
Shell Fish	*Spoiled by Acenetobacter, Moraxella* and *Vibrio.*
Chilled Shrimps	*Acinetobacter, Moraxella*, and *Vibrio*.
Crabmeat	At chilling temperature- deteriorated by *Pseudomonas, Acinetobacter, Moraxella.* At higher temperature- Proteus.
Raw Lobsters	*Pseudomonas, Alcaligenes, Flavobacterium , Bacillus.*
Oysters, and Clams	Fecal coliforms like Escherichia, fecal streptococci, *S. aureus, Salmonella enterica* serovar *enteritidis* and *typhimurium*.
Crabs and Oysters	May contain species of *Vibrio*, including *V. parahaemolyticus*
Smoked Salmon and Shrimps	Pathogenic *L. monocytogenes*
Oysters	Oysters remain in good condition as long as they are kept alive in the shell at chilling temperature, but they decompose when they are dead. Oysters are high in protein and sugars. • At temperature near freezing-*Pseudomonas, Acinetobacter, Moraxella*. • At higher temperature- souring may be the result of the fermentation of the sugars by coliform bacteria, *streptococci, lactobacilli*, and yeast to produce acid and sour odour. • Spoilage by an asporogenous yeast cause pink oyster.

NOTES

(v) Contamination and spoilage of meat products

Meat is as an ideal medium for many organisms due to the following reasons:

- **Moisture content:** Surface of the meat may be little moist to allow mold growth, still moisture to encourage yeasts, and very moist to favor bacterial growth.
- **Higher pH:** It is favourable pH for most microbial growth.
- **Nutritional content:** It is rich in nitrogenous foods, supplied with minerals and accessory growth factors. Also has some fermentable carbohydrates (glycogen).

Spoilage organisms can grow on fresh, cured, dried or cooked meats.

Spoilage in fresh meat

Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients are available.

- *Pseudomonas* sp. is the predominant spoilage bacteria in aerobically stored raw meat and poultry.
- *Shewanella putrefaciens* can produce sulfides and ammonia, which not only smell bad but also cause color changes in meat.
- *Brochothrix thermosphacta* is often a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures.
- *Enterobacteriaceae*, particularly species of *Serratia*, *Enterobacter*, and *Hafnia*, are major causes of spoilage in vacuum-packed, high pH fresh meats. These organisms are facultative anaerobes that produce organic acids, hydrogen sulfide and greening of meats.
- Lactic acid bacteria grow on meat and poultry packaged under vacuum and modified atmospheres, producing organic acids from glucose by fermentation. This gives rise to off-odors which may be accompanied by gas and slime formation and greening of meat.
- Psychrophilic, anaerobic *Clostridium* spp. are associated with spoilage of vacuum-packaged meats. 'Blown pack' meat spoilage is characterized by excessive gas formation with off odors due to formation of butyric acid, butanol and sulfurous compounds.
- Yeasts and moulds grow relatively slowly on fresh meat and do not compete well with bacteria. Therefore, they are a minor component of spoilage flora.

NOTES

Spoilage in processed meat

Spoilage organisms can grow on processed and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere.

- *Pseudomonas* spp. are not usually important causes of spoilage in processed meats because of their sensitivity to curing salts and heat pasteurization and their inability to grow well in meats packed with a vacuum or high carbon dioxide atmosphere. However, when packages have been opened and there has been insufficient curing, these bacteria may spoil refrigerated processed meats.
- Some cold- and salt tolerant *Enterobacteriaceae* have been found to cause spoilage in some specific processed meats, such as ham or bacon.
- Lactic acid bacteriaproduce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels.
- Sporeformers (*Clostridium* and *Bacillus*) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. However, faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients.
- Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or are stored aerobically in the cold.

Some of the general types of spoilage of meat is shown in Table 3.2.

Table 3.2 *General Types of Spoilage of Meats*

A. Spoilage Under Aerobic Conditions	
1. Bacterial Spoilage	
Condition	**Products Affected**
Surface Slime	Caused by species of *Pseudomona, Acinetobacter, Moraxella, Alcaligenes Streptococcus, Leuconostuoc, Bacillus* and *Micrococcus.*Temperature and the availability of moisture influence the kind of microorganisms causing surface slime. At chilling temperatures, high moisture will favor the *Pseudomonas-Alcaligenes* group; with less moistute, micrococci and yeast will be encouraged; and with still less moisture, moulds may grow.At room temperatures, micrococci and other mesophiles grow well.
Changes in Fat	The unsaturated fat in meat gets oxidized by lypolitic species of *Pseudomonas* and *Achromobacter* or by yeasts which produce off odours (rancidity) due to hydrolysis of fats and production of aldehydes and acids.
Off Odors and Off Tastes	Volatile acid like formic, acetic, butyric and propionic acid produce sour odor. Cold storage flavor or stale flavor. *Actinomycetes* produce musty or earthy flavor. Yeast also cause sliminess discoloration and off odor and taste defects.

NOTES

Change in Colour of Meat Pigments	The red colour of meat called its "bloom" may be changed to shades of green, brown or gray as a result of the production of oxidizing compounds like peroxides or by H_2S production by bacteria. *Lactobacillus and Leuconostocs* sp. cause the greening of the sausage.
Surface Colour Change	The red pigment producing bacteria, *Serratia marcescens*, caused red spots on meat. Blue color surface is caused by *Pseudomonas syncyanea* yellow discoloration is caused by *Micrococcus* or *Flavobacterium*. *Chromobacterium lividum* give greenish-blue to brownish-black spots on stored beef. The purple "stamping ink" discoloration of surface fat is caused by yellow pigmented cocci and rods. When the fat become rancid and peroxides appear the yellow color changes to greenish shade and later becomes purplish to blue.
Yeast Spoilage: Under aerobic condition yeasts may grow on the surface of meats, causing sliminess, lipolysis, off-odors and tastes, and discoloration- white, cream, pink or brown –due to pigments in the yeast.	
Aerobic Growth of Moulds may cause stickiness, whiskers (when meat is stored near freezing temperature, a limited amount of mycelia growth may take place without sporulation. Such a fuzzy white growth can be caused by a number of moulds including *Thamnidium chaetocladiodes, Mucor, Rhizopus*), black-spot (*Cladosporium herbarum*), white-spot (*Sporotrichum carnis*), green patches (species of *Penicillium*), off odor and off taste.	
B. **Spoilage Under Anaerobic Condition**	
Souring	Sour odor and taste caused by production of formic, acetic, butyric, propionic and higher fatty acids or other fatty acids such as lactic or succinic. Souring can occur by a) action of the meat's own enzymes during aging or ripening b) anaerobic production of fatty acids or lactic acids by bacterial action c) proteolysis without putrefaction caused by facultative or anaerobic bacteria and sometimes called 'stinking sour fermentation'.
Putrefaction	True putrefaction is the anaerobic decomposition of proteins with the production of foul smelling compounds like hydrogen sulphide, mercaptans, indol, scatol, ammonia and amines. It is usually caused by species of *Clostridium*, but facultative bacteria also may cause or assist in putrefaction such as *Pseudomonas and Alcaligenes (P. puterfaciens). Proteus* are also putrefactive.
Taint	Inexact word applied to any off-taste or off-odor. The term 'bone taint' of the meat refers to either souring or putrefaction next to the bones, especially in hams. Usually it means putrefaction.

(vi) Contamination and spoilage of eggs

There are several factors that protect eggs from spoilage, which are as follows:

- The outer waxy shell membrane; the shell; and the inner shell membrane, each is effective to some degree in retarding the entry of microorganisms.
- Internally, lysozyme is present in egg white, which is quite effective against Gram-Positive Bacteria.
- Egg white also contains avidin, which forms a complex with biotin, thereby making this vitamin unavailable to microorganisms.
- In addition, hen egg albumen contains ovotransferrin, which chelates metal ions, particularly Fe^{3+}, and ovoflavoprotein, which binds riboflavin. At its normal pH of 9–10, egg albumen is cidal to Gram-positive bacteria and yeasts at both 30°C and 39.5°C.

There are several factor that promote spoilage, which are as follows:

- The nutrient content of the yolk material.
- pH in fresh eggs (about 6.8) make it an excellent source of growth for most microorganisms.

NOTES

Table 3.3 shows the spoilage of eggs by Bacteria and Moulds.

Table 3.3 *Spoilage of Eggs by Bacteria and Moulds*

<table>
<tr><td colspan="5">A. Spoilage of Eggs by Bacteria

Fresh eggs are generally sterile particularly the inner contents. However, breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter in to the egg yolk and cause spoilage on storage.</td></tr>
<tr><td colspan="5">1. Different Types of Rots</td></tr>
<tr><td>Green Rot caused by Pseudomonas fluorescence, a bacterium that grows at 0 degree C. The white of egg is bright-green color. This stage is noted with difficulty in candling but visible when the egg is broken. The content of egg fluoresce strongly under ultraviolet light.</td><td>Colorless Rot caused by Pseudomonas, Acinetobacter, Alcalegenes, certain coliform bacteria. These dots are detected readily by candling, for the yolk usually is involved, except in very early stages, disintegrates or at least shows a white incrustation. The odor varies from a scarcely detectable one to fruity to highly offensive.</td><td>Black Rots caused by many species of Proteus, some species of Pseudomonas and Aeromonas. Eggs are almost opaque to the candling lamp, because the yolk become blackened and then break down to give the whole egg contents a muddy brown color and also there is strong putrid odor due to the formation of H_2S.</td><td>Pink Rots are caused by strains of Pseudomons and may at times be a later stage of some of the green rots. They resemble the colorless rots, except for a pinkish precipitate on the yolk and a pink color in the white.</td><td>Red Rots are caused by species of Serratia, are mild in odor and are not offensive</td></tr>
<tr><td colspan="5">The development of black rot and red rot usually means that the egg has at some time been held at temperatures higher than those ordinarily used for storage.</td></tr>
<tr><td colspan="5">2. Off-Foavors developed by bacteria such as Achromobacter perolens, Pseudomonas graveolens and P mucidolens. A hay odor is caused by Enteribactor cloacae, while fishy flavors are produced by certain strains of Escherichia coli.</td></tr>
<tr><td colspan="5">B. The Spoilage of Eggs by Fungi</td></tr>
<tr><td colspan="5">In storage atmosphere of high humidity a variety of moulds may cause superficial fungal spoilage, first in the form of a fuzz or whiskers covering the shell and later as more luxiarant growth.</td></tr>
<tr><td colspan="5">Egg are stored usually near-freezing temperature, which is high enough for slow mycelial growth of some moulds but too low for sporulation, while other moulds may produce asexual spores. Moulds causing spoilage of eggs include species of Penicillium, Cladosporium, Sporotrichium, Mucor, Thamnidium, Botrytis, and Alternaria.</td></tr>
</table>

Spoilage of eggs by fungi goes through stages of mold growth

The spoilage of egg by fungi occur in the follwing stages:

1. Very early mold growth is termed pin-spot molding because of small, compact colonies of mold appearing on the shell and usually just inside it. The color of these pin spots varies with the kind of mold spoiling the egg; *Penicillium* species cause yellow or blue or green spots inside the shell. *Cladosporium* species give dark-green or back spots, and species of *Sporotrichium* produce pink spots.
2. The final stage of spoilage by moulds is fungal rotting, after the mycelium of the mold has grown through the pores or cracks in the egg. Jellying of the white may result and colored rots may be produced, for example fungal red rot by *Sporotrichium* and a black color by *Cladosporium.*
3. The hyphae of the mold may weaken the yolk membrane enough to cause it is rupture after which the growth of the mold is stimulated greatly by the food released from the yolk.

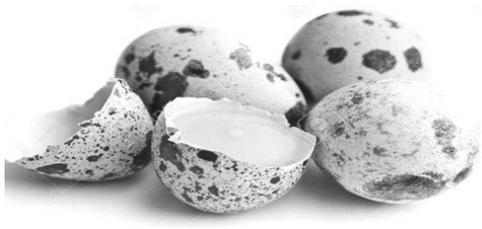


Fig. 3.5 *Spoilage of Egg*

(vii) Contamination and spoilage of poultry

Approximately 31% of meat consumed worldwide is poultry. The two principal types of poultry meat consumed are chicken and turkey. The shelf life of poultry depends on a combination of factors, including the number and types of microorganisms initially present, storage temperature, atmosphere and packaging conditions. The chilling process before packaging, whether by air or water, influences the type and number of contaminants on the carcass in the package. It is important to freeze the poultry fast in order to keep it in good condition for several months. Freezing further reduces the number of microorganisms in the poultry meat provided the temperature is maintained quite low (-18°C or below).

Most bacterial growth takes place on the surfaces, i.e., the skin, the lining of the body cavity, and any cut surfaces, and the decomposition products diffuse slowly into the meat. Isolates from poultry and poultry products include members of numerous genes.

Table 3.4 *Microbes Involved in Spoilage of Poultry*

Microbes Isolated from Poultry	
Product	**Microorganisms Isolated**
Incoming Birds	**Bacteria** *Acinetobacter, Moraxella, Pseudomonas, Flavobacterium, Staphylococcus, Micrococcus*
After Processing	**Bacteria** *Acinetobacter, Moraxella, Pseudomonas, Flavobacterium, Staphylococcus, Micrococcus, Cytophage, Enterobacter, Alcaligenes, Salmonella, Campylobacter* **Yeast** *Trichosporon, Torulopsis, Candida, Rhodotorula* **Moulds** *Penicillium, aaaspergillus, Alternaria*
Microbes Involved in Spoilage of Refrigerated Poultry	
Raw Eviscerated Carcasses Held at 10 degree C Or Below Above, 10 degree C	**Bacteria** Spoiled mostly by bacteria, such as *Pseudomonas fluorescens, P. putida, Acinetobacter, Moraxella.* **Yeast** Spoiled less extent to *Torulopsis* and *Rhodotorula* micrococci usually predominate, there also is growth of *Alcalegenes and Flavobacterium.*
Dark Meat, pH 6.4-6.7	*Acinetobacter, Alteromonas, Pseudomonas.*
White Meat, pH 5.7-5.9	*Pseudomonas* and others.
Chicken Wrapped in Oxygen-Impermeable Films	*Microaerophilic* bacteria, lactic acid bacteria, and others.
Vacuum-Packed Chicken	*Enterobacter* and others.
Iced, Cut-Up Poultry	Often develops a slime accompanied by an odor described as tainted, acid, sour, and dishraggy. This effect is caused chiefly by species of *Pseudomonas*, *Alcalegenes* also may be concerned.

NOTES

(viii) Contamination and spoilage of canned food

Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container made up of tin (tin can), wrought iron canisters. The idea is to make food available and edible long after the processing time. This is an important method of packaging food for long term storage. Normally food is stored in metallic containers along with heat treatment. The heat treatment varies depending upon type of food. There is always a chance that microorganisms may survive if the heat treatment is not proper thereby leading to spoilage of food. Although the canning process is usually designed to provide a sterile product, spoilage in these products does occur as a result of:

- Preprocess spoilage
- Inadequate processing
- Post-processing contamination

To prevent the food from being spoiled before and during containment, a number of methods are used: pasteurisation, boiling (and other applications of high temperature over a period of time), refrigeration, freezing, drying, vacuum treatment, antimicrobial agents that are natural to the recipe of the foods being preserved, a sufficient dose of ionizing radiation, submersion in a strong saline solution, acid, base, osmotically extreme (for example very sugary) or other microbially-challenging environments.

Causes of spoilage in canned foods

Usually the incidences of food spoilage in cans are low. The spoilage of can could be due to biological or chemical reasons or combination of both. The biological spoilage is primarily due to microbial growth while chemical spoilage is due to hydrogen produced due to reaction of acid in food and iron on can. The degree of swelling can also be increased by high summer temperature and high altitudes. Certain other factors, such as overfilling, buckling, denting or closing the can while cool may also be responsible for spoilage of foods in cans.

Types of spoilage

The types of spoilage are as follows:

(a) Chemical spoilage

The chemical spoilage in most cases is due to production of hydrogen gas produced in can because of action of acid of food on iron of can. This spoilage is termed as Hydrogen swell. It occurs due to following factors:

- Increased storage temperature
- Increased acidity of food
- Improper exhaust
- Presence of soluble sulfur and phosphorous compounds
- Improper timing and lacquering of can at internal surfaces

(b) Biological spoilage

The cause of biological spoilage is microbial activity. In heat treated cans, the growth of microorganisms occur due to the following reasons:

NOTES

- **Leakage of can:** It occurs because of manufacturing defects, punctures or rough handling. Bacteria are introduced into can by either in holes or improper seams. In this type, the microorganisms are not usually heat resistant and wide array of organisms had been found to cause spoilage as it is *post processing contamination*. Microbes may also get entry into can due to cold water, used to cool cans after heat treatment. Leakage may also be responsible for release of vacuum, which can favor the growth of microorganisms.
- **Survival of organisms after the administration of heat treatment:** It includes sub-optimal heat treatment, faulty retort operations, excessive microbial load and contamination in product, change in consistency of the product.

(c) Microbial spoilage

It occurs due to the action of microbes.

Spoilage by thermophilic spore forming bacteria: Spoilage by these types of bacteria is most prevalent in under processed heat treated canned foods. Their spores survive the heat treatment and undergo vegetative cell formation and subsequent growth in canned conditions. The spores of thermophilic bacteria do not germinate to cause spoilage in heat-treated canned products stored at 30°C or below. If can temperature increases to 40 °C or high, the spores germinate, multiply, and spoil the canned food.

- **Flat sour spoilage**

This is caused by souring bacteria. Name is derived from the fact that the ends of can remain flat during souring, or the development of lactic acid in the food by the flat sour bacteria. Gas is not produced (therefore flat), only acid is produced. Because the can retains a normal outward appearance, the detection of spoilage from outside is not possible thereby culturing of contents become necessary to detect the type of organisms. Occurs more frequently in low acid foods, such as peas and corns and is caused by *Bacillus stearothermophilus* (spores of thermophiles are more heat resistant and may serve the heat process to cause flat sour spoilage). Flat sour spoilage of acid foods, such as tomatoes or tomato juice is caused by facultative thermophilic *Bacillus coagulans.* It can not bulge (flat) but pH falls and food tastes sour, off odors may be apparent.

- **TA spoilage**

The bacterium causing this type of spoilage has been nick named TA, which is short for thermophilic anaerobe not producing H_2S or for the species of C*lostridium thermosaccharolyticum.* It produces acid and gas in foods. The gas, a mixture of CO_2 and H_2, swells the can. Spoiled food produces sour or cheesy smell. Blackening of the food may also be observed.

- **Sulfide or sulfur stinker spoilage**

It caused by *Desulfotomaculum nigricans*is. The spores of this bacterium have considerably less heat resistance than those of flat sour and TA bacteria, hence their appearance in canned foods is indicative of gross underprciessing. This type

NOTES

of spoilage occurs in low acid foods and H_2S, formed in canned peas or corn, is evident by odor when the can is opened. In corn, a bluish gray liquid is evident in which blackened germs and grey kernels of corn float. Peas usually give the H_2S odour but without any TA bacteria, but manure can also be an original source.

• Spoilage by mesophillic spore formers

Spoilage by mesophilic microorganisms results from underprciessing and is caused by spore forming bacteria of the genera Clostridium and Bacillus

Spoilage by mesophilic Clostridium species

Species of *Clostridium* may be sugar fermenting, for example *C. butyricum* and *C. pasteurianum* and cause by butyricum acid type of fermentation in acid or medium acid foods, with swelling of the container by the carbon dioxide and hydrogen gas produced. Because of the spores of clostridia have a comparatively low heat resistance, spoilage by these anaerobes take place most commonly in canned foods which have been processed at 100 degree C or less. Thus canned acid foods, such as pineapple, tomatoes, and pears are spoiled by *C. pasteurianum*. Such spoilage is more likely when the pH of the food is above 4.5. The putrefactive anaerobes grow best in the low acid canned foods, such as peas, corns, meat, fish, poultry, bit on rare occasion may spoil other foods.

Spoilage by mesophilic bacillus species

Spores of *B subtilis and B mesentericus* survive in low acid home canned foods that had been given a heat processing at 100 degree C. Commercial canned foods have been spoiled by *Bacillus* species, especially in poorly evacuated cans. Foods so spoiled have been mostly canned sea foods, meats and evaporated milks. Entrance may have been through a leak in the container. Spores of these bacteria have about the same heat resistance as those of *Clostridium pasteurianum.*

• Spoilage by non-spore forming bacteria

Presence of non-spore formers in cans indicate *post processing contamination* or the bacteria entered the container through a leak. Vegetative cells of some kind of bacteria are fairly heat resistant in that they can withstand pasteurization. Among these thermoduric bacteria are the enterococci, *Streptococus thermophilous*, some species of *Micrococcus* and *Lactobacillus* and *Microbacterium.* Acid forming *Lactobacillus* and *Leuconostoc* species has been found growing in underproceessed tomato products, pears and other fruits. Micrococci have been reported in meat paste and in similar product with very poor heat penetration and *S. faecalis*, or *S. faecium* is often present in canned hams that are only partially sterilized and may be responsible for spoilage on storage.

• Spoilage by fungi

It includes the spoilage by yeasts and moulds. Yeasts and their spores are not thermo tolerant, thus they are not found in suitably heat treated cans. Their presence indicates under processing or post pasteurization contamination through leakage. Fermentative yeasts are more prominent and they produce carbon dioxide, thus causing swelling of cans. Film yeasts too can grow on the surface of the food products.

NOTES

On other hand, moulds includes *Aspergilus* and *Penicillium* are most spoiling organisms. These can grow at high sugar concentration. Acidification is considered method of preventing growth of moulds. Some of the moulds are resistant to heat. Moulds are more common in home canned foods where heating as well as sealing is not under total aseptic conditions.

Fig. 3.6 *Swelling of Can and a Normal Can*

Check Your Progress

4. What do you understand by an adulterant?
5. What is the common adulterant for coffee seeds?
6. Name the common bacterial genera spoiling grains.
7. How does fish show discolouration during spoilage?

3.4 MICROSCOPIC EXAMINATION OF FOOD FOR ADULTERANTS

Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if food is improperly cooked or stored. Specifically those food items that are used to produce fermented foods, such as cheese, yogurt, bread, beer, and wine, and those with other useful roles, such as producing probiotics.

As per the definition, the term 'Microbiology' refers to a branch of biology that deals with the study of microorganisms and their different activities. Since ages, these microorganisms are playing a potential role in human welfare both as useful and harmful biological agents. This leads to an extensive study of these micro-organisms with the aim of understanding their growth and nutritional requirements for their production or destruction both. Food safety is a key emphasis of food microbiology. Numerous agents of disease, pathogens, are readily transmitted via food, including bacteria, and viruses. Microbial toxins are also possible contaminants of food. However, microorganisms and their products can also be used to combat these pathogenic microbes. The bacteriophages, viruses that only infect bacteria, can be used to kill bacterial pathogens.

NOTES

Microorganisms are living organisms that are individually too small to see with the naked eye. The unit of measurement used for microorganisms is the micrometer (μm); 1 mm = 0.001 millimeter; 1 nanometer (nm) = 0.001 mm. Microorganisms are found everywhere (ubiquitous) and are essential to many of our planets life processes. With regards to the food industry, they can cause spoilage, prevent spoilage through fermentation, or can be the cause of human illness. There are numerous classes of microorganisms, of which bacteria and fungi (yeasts and moulds), the bacterial viruses or bacteriophage are the most common forms.

Let us study some of the tests to examine food adulteration in detail.

(A) Resazurin Reduction Test (RRT)

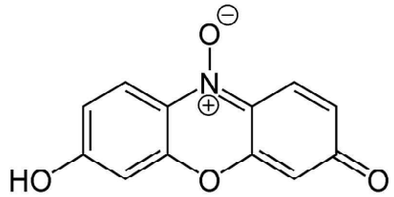
In this test, the Resazurin Dye undergoes reduction through a series of colour shades, such as blue, purple, lavender, and pink before finally getting reduced to colourless (white). This colour change is dependent upon the extent of depletion of oxygen by metabolic activity of microbes. Resazurin dye which is blue in colour (redox potential of +0.3 volts) undergoes an irreversible change to Resorufin (redox potential +0.2 volts) a pink colour compound. Further redox potential reduction (+0.1 volts or less), the dye colour changes to dihydroresorufin, a colourless form in a reversible reaction. The colour change is measured with the help of a lovibond colour comparator and a standard resazurin disc.

Chemical structures and molecular formulas of resazurin and resorufin are follows:

Resazurin

The molecular formula of resazurin is - $C_{12}H_7NO_4$

Its chemical structure is as follows:

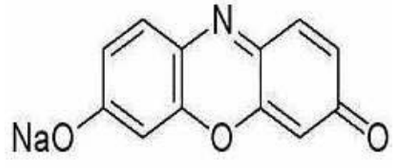


Resazurin (7-Hydroxy-3H-Phenoxazin-3-one 10-oxide)

Resorufin

The molecular formula of resorufin is - $C_{12}H_7NO_3$

Its chemical Structure of as follows:



Resorufin (7-Hydroxy-3H-Phenoxazin-3-one, Sodium Salt)

NOTES

Materials required

***Milk Sample*:** Raw and pasteurized milk

***Reagents*:** Resazurin solution 0.05%, lovibond colour comparator

***Equipment*:** Resazurin colour disc, water bath at 37.5°C, screw cap tubes 10 ml, pipettes 10 ml and 1 ml

Resazurin dye

To make a Resazurin dye solution of 0.05%, dissolve 0.05 G of resazurin powder in 100 ml distilled water and boil for 30 minutes. Standard solution should be stored in dark bottle at cool place. To make working solution of 0.005%, Dilute 1 ml of standard solution with 10 ml of distilled water before use.

Procedure

To perform the test, follow the given steps:

1. Take 10 ml of each milk sample into a sterile screw cap tube.
2. Add 1 ml of resazurin solution.
3. Cap the tubes to prevent oxygen entry.
4. Mix the solution by inverting the tubes 2-3 times.
5. Place the test tubes in a water bath at 37^{o}c and incubate for 1 hour.
6. Take the tube out after the incubation and match the colour of the milk with one of the colour standards of resazurin disc.

Result

It is show in Table 3.5.

Tabale 3.5 *Result of RRT*

Colour of Sample	Quality of Milk
1. Blue (No Colour Change)	Excellent
2. Lilac	Very Good
3. Mauve	Good
4. Pink Mauve	Fair
5. Mauve Pink	Poor
6. Pink	Bad
7. White	Very Bad

Advantages

Some of the advantages of the test are as follows:

- Test is cheaper and can be measured in a shorter time.
- Microbial activity is measured rather than the number of bacteria.

NOTES

Disadvantages

Some of the disadvantages of the test are as follows:

- Continuous observation is required until reduction takes place.
- Not suitable for milk samples with low bacterial counts (less than 10^5/ml).
- Does not indicate for the type of microorganisms present in the milk sample.

(B) Alkaline Phosphatase Test (ALP)

Alkaline Phosphatase (ALP) is a heat sensitive enzyme in milk that is used as indicator of pasteurization. If milk is properly pasteurized, Alkaline Phosphatase is inactivated. Alkaline Phosphatase induces the Hydrolysis of p-Nitrophenyl Phosphate at Alkaline pH, and liberates para-Nitrophenol, a yellow colored complex whose intensity is directly proportional to the concentration of ALP in the sample, and can be measured at 405 nm. The colour intensity is measured by direct comparison with standard colour discs in a Lovibond comparator.

Materials required

Test tubes, pipettes – 1 ml, 5 ml and 10 ml, water bath at 37°c, lovibond comparator discs of standard for nitrophenol, p-nitrophenyl phosphate (pnpp) disc

Reagents

1. p-Nitrophenyl Phosphate (pNpp) solution
2. **Sodium carbonate-bicarbonate buffer:** Dissolve 3.5 G of anhydrous sodium carbonate and 1.5 G of sodium bicarbonate in one litre of distilled water.
3. **Buffer substrate:** Dissolve 1.5 g of disodium p-nitrophenyl phosphate in one litre of sodium carbonate-bicarbonate buffer. This solution is stable if stored in a refrigerator at 4°c or less for one month but a colour control test should be carried out on such stored solutions.

Procedure

To perform the test follow the given steps:

1. Pipette 5 ml of buffer substrate into two clean, dry test tubes.
2. Add 1 ml of the raw milk to be tested in one and pasteurized milk in second tube for control.
3. Stopper the tubes and invert mix 2-3 times.
4. Place the tubes in the water bath and incubate for 30 minutes.
5. Remove the tubes from the water bath and invert mix each tube.
6. Observe/read the colour developed or match the colour of the milk with one of the colour standards of pnpp disc.

Result

Development of yellow colour in the tubes indicate bad quality of milk whereas white unchanged colour of milk after incubation indicates good quality.

Check Your Progress

8. Define food microbiology.
9. What is the factor responsible for the colour change when resazurin dye undergoes reduction?
10. Write the molecular formula of resazurin.

NOTES

3.5 PESTICIDE ANALYSIS IN FOOD PRODUCTS

The pesticides are used to increase the production of food, cotton fiber and tobacco and to control many vector-borne diseases such as malaria and typhus. However, many of these pesticides are extremely dangerous and exposure to them can result in adverse health problems. According to a joint report from the Food and Agriculture Organization (FAO) and UNEP, approximately 30% of pesticides marketed in developing countries do not conform to international standards, contain active ingredients exceeding toxic thresholds, and do not exclude other toxic substances. According to WHO, an estimated 1–5 million cases of pesticide poisoning occur every year among agricultural workers and result in 20000 fatalities, most of these are in developing countries. Some of the most commonly used hazardous pesticides that are said to be currently in use include aldicarb, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, carbofuran, cyfluthrin, dichlorvos, methomyl, parathion, methamidophos, alphacypermethrin, deltamethrin, endosulfan, etc. Typically, human exposure to pesticides used in agriculture is highest among farm workers, pesticide appliers and those who live adjacent to heavily treated agricultural land. In addition to being frequently exposed to a wide array of pesticides, farm workers and pesticide appliers are likely to be exposed to high doses by multiple routes. Misuse of highly toxic pesticides, a lack of attention to safety precautions, poor spraying techniques and inadequate personal protection during pesticide use are some of the main reasons for the high incidence of pesticide intoxication observed. Indiscriminate use of pesticides represents one of the main environmental and public health problems globally, contributing to soil contamination, water pollution, destruction of useful organisms and development of pesticide resistance in pests, and consequently leading to harmful effects on the health of both farmers and food consumers. The typical pesticides detected in soil, water and crops include organochlorines such as DDT, endosulfan and lindane. For example, hazardous pesticides including DDT and its breakdown products endosulfan I and II, endosulfan sulfate and profenofos were detected in the soil samples collected from various agro farming sources. Similarly, residues from six banned or restricted chemical pesticides – DDT, endosulfan, lindane, aldrin, dieldrin and endrin – were reported in food samples as well. Humans are substantially exposed to DDT and DDE through indoor spraying. Such exposure may result in a range of health effects, including reduced fertility, genital birth defects, breast cancer, diabetes and damage to the developing foetus's brain. DDE is known to also block male

NOTES

hormone activity. DDT is one of the 12 original POPs banned under the Stockholm Convention, but its use in the control of malaria and other vector-borne diseases has been granted a "health-related exemption" by the Parties to the POPs treaty until cost-effective substitutes for malaria control are found since cessation of DDT use caused catastrophic epidemics of malaria in several parts of the world. Organophosphate-chlorinated hydrocarbons are detected in the majority of pesticide analyses. Carbamate residues are another major topic of investigation, as producers increasingly use these pesticides as alternatives to organophosphates.

3.5.1 Extraction and Purification of Sample

Separation techniques are those techniques that can be used to separate two different states of matter, such as liquid and solid. Such separation techniques include filtration or evaporation. Separation process, or a separation method, or simply a separation, is a methodology to attain any mass transfer phenomenon that converts a mixture of substances into two or more distinct product mixtures. The methods for separating, collecting, and detecting radionuclides are similar to ordinary analytical procedures and employ many of the chemical and physical principles that apply to their nonradioactive isotopes. However, some important aspects of the behavior of radionuclides are significantly different, resulting in challenges to the radiochemist to find a means for isolation of a pure sample for analysis.

A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct product mixtures. At least one of results of the separation is enriched in one or more of the source mixture's constituents. In some cases, a separation may fully divide the mixture into pure constituents. Separations exploit differences in chemical properties or physical properties, such as size, shape, mass, density, or chemical affinity between the constituents of a mixture.

Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis. Proteins can be purified based on characteristics, such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase. Four separation techniques based on molecular characteristics and interaction type use mechanisms of ion exchange, surface adsorption, partition, and size exclusion. Other chromatography techniques are based on the stationary bed, including column, thin layer, and paper chromatography. Column chromatography is one of the most common methods of protein purification.

3.5.2 High Performane Liquid Chromatography (HPLC)

It is an improved or advanced form of column chromatography. It can be used in various forms depending on the stationary phase. It may be normal phase HPLC, reverse phase HPLC, ion exchange HPLC or size exclusion HPLC. It is useful in separation, purification, analysis of structural, analysis of functional aspects and quantification. This technique is fast and efficient. HPLC can be used effectively for purification and analysis of amino acids, lipids, carbohydrates, nucleic acids, proteins, steroids, and other biologically active molecules. The results can be

obtained in very short time. HPLC is used in a variety of industrial and scientific applications including pharmaceutical, environmental, forensics, and chemicals. The general instrumentation for HPLC includes pump, injector, column, detector, data acquisition and display (Refer Figure 3.7).

NOTES

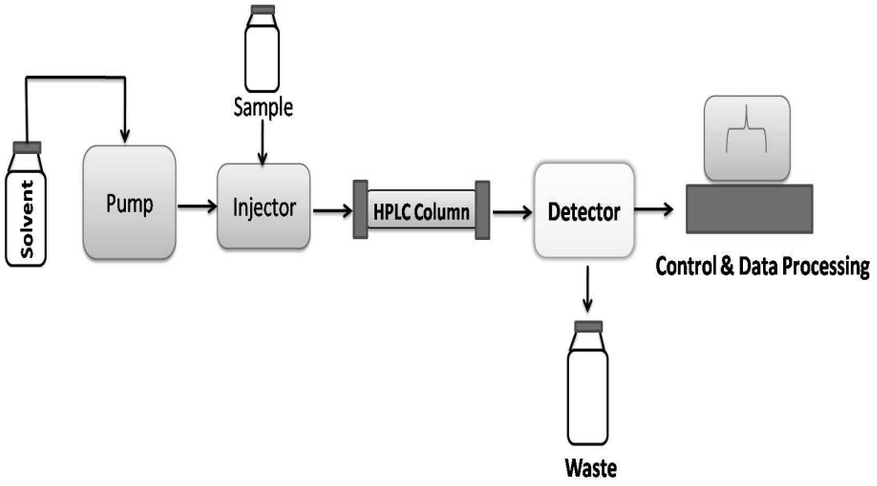


Fig. 3.7 *General Instrumentation or Setup for HPLC*

The stationary phase should be resistant to mechanical and chemical damage. High pressure is applied in HPLC to derive the solvent through packed column. In HPLC, sample mixture or analyte in a solvent (known as the mobile phase) is pumped at high pressure through a column having suitable chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. Through HPLC, the trace concentrations of compounds in a sample can be separated and identified. The HPLC operate with basic principle similar to other chromatographic techniques. Separation of a sample into its constituent parts depends on the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

3.5.3 Gas Chromatography for Organophosphates

It is a gas-liquid chromatography. It is a commonly used technique for identification, purification, quantification and analysis of compounds in a sample. GC is used in various quality control processes. Gas chromatography is a simple, multifaceted and highly sensitive technique. Molecules in very small amount can be separated with this technique. Gas chromatography is also used to monitor industrial processes. A large variety of samples can be analyzed as long as the compounds are sufficiently thermally stable and reasonably volatile. In gas chromatography, stationary phase consists of a packed column. The column contains an inert solid support coated with liquid stationary phase. Its mobile phase or carrier phase consists of gases like helium, argon, or nitrogen. Theses gases are known as carrier gases. The mobile phase (inert gas) is passed through the column under high pressure. Liquid samples are vaporized before injection into the carrier stream. The vaporized sample enters the gas stream which transports the sample into a separation column. The various components are separated inside this column. The attached detector measures the quantity of the components that exit the column.

NOTES

The concentration of sample can be determined by using a standard sample with known concentration. The peak retention time (appearance time) and area of standard sample are used for calculation of test sample concentration.

3.5.4 Thin-Layer Chromatography for Identification of Chlorinated Pesticides

It is a solid-liquid adsorption chromatography. Thin layer chromatography is used to separate and analyze complex biological or non-biological samples into their constituents. It is also used for testing the purity of a sample. In thin layer chromatography, the stationary phase consisted of solid adsorbent substance coated on glass plate. The solid adsorbent material can be based on silica or alumina. In general laboratory experimentation, silica coated on glass plate is used for performing TLC. The sample is applied in form of spot and allowed to run in presence of suitable mobile phase (solvent). The mobile phase (solvent) runs from bottom towards top by diffusion. The sample travels along with the mobile phase and get distributed on the stationary phase and solvent. The different molecules are separated on the stationary phase due to different mobility. The movement depends on nature of the substance, functional groups on substance, polarity of the substance, stationary phase, solvent system, functional groups on stationary phase and other factors. If the components in sample are colorless, then suitable chemical substance or fluorescence can be used for development of color of the separated molecules. The developed color can be checked by visible light or UV light. The position of each molecule in the mixture can be measured by calculating the ratio between the distances travelled by the molecule and the solvent. The ratio is denoted by R_f.

Check Your Progress

11. What does HPLC stand for?
12. Which technique is used to monitor industrial processes?

3.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Food analysis is the study of analytical processes for describing the qualities of foods and their ingredients, as well as their creation, application.
2. Any inorganic particle detected in food is referred to as ash.
3. Phosphorus also assists in the removal of waste and the restoration of damaged tissues.
4. Any material which is or could be employed for the purposes of adulteration is known as adulterant.
5. Coffee seeds are contaminated with tamarind seeds, mustard seeds, and chicori.
6. Common bacterial genera spoiling grains are Pseudomonas, Micrococci, Lactobacillus and Bacillus.

NOTES

7. Discoloration of the fish flesh may occur during spoilage as follows:
   - Yellow to greenish yellow colors caused by Pseudomonas fluorescence, yellow micrococci.
   - Red or pink colors from growth of Sarcina, Micrococcus, Bacillus sp. or by moulds or yeasts.
   - Chocolate brown color by Asporogenous yeast.
8. Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if food is improperly cooked or stored
9. The resazurin dye undergoes reduction through a series of colour shades, such as Blue, Purple, Lavender, and Pink before finally getting reduced to Colourless (White). This colour change is dependent upon the extent of depletion of oxygen by metabolic activity of microbes.
10. The molecular formula of resazurin is $C_{12}H_7NO_4$.
11. HPLC stands for High Performance Liquid Chromatography.
12. Gas chromatography is also used to monitor industrial processes.

3.7 SUMMARY

- The types and proportions of nutrients present in the food are all important in determining what organisms are most likely to grow.
- Prevention of food adulteration act of 1954 has been in force since 1 June 1955.
- It has been estimated that one-fourth of all fruit produce harvested is spoiled before consumption.
- As soon as fruits and vegetables are gathered into boxes, lugs, baskets, or trucks during harvesting, they are subject to contamination with spoilage organisms from each other and from the containers unless these have been adequately sanitized.
- Sorting spoiled fruits or vegetables or trimming spoiled parts removes microorganisms, but additional handling may result in mechanical damage and therefore, greater susceptibility to decay.
- In the processing plant, fruits and vegetables are subjected to further contamination and chances for growth of microorganisms, or numbers and kinds of organisms may be reduced by some procedures.
- Inclusion of decayed parts of fruits increases the numbers of microorganisms in fruit juices.
- Harvested grains are infected by microorganisms from soil and insects. Grains are infected by bacteria which belong to Pseudomonadaceae and Lactobacillaceae.

NOTES

- There are different types of cereal products, namely whole cereals, milled grain products, processed cereals and ready mixes.
- Common bacterial genera spoiling grains are Pseudomonas, Micrococci, Lactobacillus and Bacillus.
- The process of flour making such as washing and the milling reduce the microbial content and hence microbial load. Moisture content of less than 15% does not allow growth of moulds.
- Fish is a very perishable food due to its high nutritional content.
- Many molluscan shellfish (primarily oysters, clams, and mussels) are customarily consumed raw or with minimal heating and have caused foodborne illness outbreaks and mortality.
- Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants.
- Spoilage organisms can grow on processed and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere.
- The two principal types of poultry meat consumed are chicken and turkey.
- Most bacterial growth takes place on the surfaces, i.e., the skin, the lining of the body cavity, and any cut surfaces, and the decomposition products diffuse slowly into the meat.
- Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container made up of tin (tin can), wrought iron canisters.
- Spoilage by mesophilic microorganisms results from underprciessing and is caused by spore forming bacteria of the genera Bacillus and Clostridium.
- 'Microbiology' refers to a branch of biology that deals with the study of microorganisms and their different activities.
- Numerous agents of disease, pathogens, are readily transmitted via food, including bacteria, and viruses. Microbial toxins are also possible contaminants of food.
- The two tests used for pesticide analysis are the resazurin reduction test and alkaline phosphatase test.
- Separation techniques are those techniques that can be used to separate two different states of matter, such as liquid and solid. Such separation techniques include filtration or evaporation.
- A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct product mixtures.
- A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct product mixtures.
- High Performance Liquid Chromatography (HPLC) is an improved or advanced form of column chromatography. It can be used in various forms depending on the stationary phase.

- Gas chromatography is a gas-liquid chromatography. It is a commonly used technique for identification, purification, quantification and analysis of compounds in a sample.

  Thin layer chromatography is a solid-liquid adsorption chromatography. Thin layer chromatography is used to separate and analyze complex biological or non-biological samples into their constituents.

3.8 KEY TERMS

- **Adulteration:** It refers to the action of making something poorer in quality by the addition of another substance.
- **Adulterant:** It refers to a material/substance which is or could be employed for the purposes of adulteration.
- **Chromatogrpahy:** Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- **Pesticide Analysis:** It refers the testing of the chemicals such as pesticides, pharmaceuticals, food additives from the source to the final target. It is imperative to perform to comprehend, their impact on the environment.
- **High Performance Liquid Chromatography (HPLC):** It is an improved or advanced form of column chromatography. It can be used in various forms depending on the stationary phase.
- **Gas Chromatography**: It is a commonly used technique for identification, purification, quantification and analysis of compounds in a sample.
- **Thin Layer Chromatography:** It is a solid-liquid adsorption Chromatography which is used to separate and analyse complex biological or non-biological samples into their constituents

3.9 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. State the advantages and disadvantages of the resazurin reduction test.
2. What are the different adulterants in food?
3. When does an article of food called adulterated?
4. How does the thin-layer chromatography identify chlorinated pesticides?

Long-Answer Questions

1. Describe the different components of food on which microorganisms require energy in detail.
2. Discuss factors responsible that promote spoilage from egg.

3. Analyse various causes of spoilage in canned foods.
4. Describe the procedure of the alkaline phosphatase test in detail. Also, mention its result.

NOTES

3.10 FURTHER READING

Dash, Dhruba Charan. 2011. *Analytical Chemistry*. New Delhi: Prentice Hall India Pvt, Limited

Hibbert, D.B. and J.J. Gooding. 2006. *Data Analysis for Chemistry*. UK: Oxford University Press.

Benwart, G. J. 1987. *Basic Food Microbiology*. New Delhi: CBS Publishers & Distributors.

Christian, Gary D. 2007. *Analytical Chemistry*, 6th Edition. Noida: Wiley India Pvt. Limited

UNIT 4 ANALYSIS OF WATER POLLUTION

NOTES

Structure

4.0 INTRODUCTION

Water pollution (also known as aquatic pollution) is the polluting of water bodies, usually as a result of human activity, in such a way that its lawful uses are harmed. Pollution diminishes a body of water's ability to deliver ecosystem services that it might otherwise give. Lakes, rivers, oceans, aquifers, reservoirs, and groundwater

NOTES

are examples of water bodies. When toxins are injected into these bodies of water, contamination occurs. Water pollution can cause water-borne diseases in humans, in addition to causing damage to numerous animals. This unit will discuss sources and types of wastewater along with the importance of water analysis and its parameters. In addition, it will explain various heavy metals and their health significance. Also, it will describe instrumental techniques for the analysis of heavy metals in aqueous systems. It will also introduce the concepts of dissolved oxygen, biochemical oxygen demand and chemical oxygen demand. It will also explain the role of pesticides as water pollutants and different water laws and standards.

4.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the sources and types of wastewater
- Explain the importance of water analysis and its parameters
- Discuss heavy metals and their health significance
- Describe instrumental techniques for the analysis of heavy metals in aqueous systems
- Evaluate measurements of dissolved oxygen, biochemical oxygen demand and chemical oxygen demand
- Understand the role of pesticides as water pollutants
- Discuss different water laws and standards

4.2 WASTEWATER: ORIGIN AND TYPES

Wastewater is water whose physical, chemical or biological properties have been changed as a result of introduction of certain substances which render it unsafe for some purposes such as drinking. The day-to-day activities of humans are mainly water dependent and therefore they discharge 'waste water' into water. Some of the substances include body wastes (faeces and urine), shampoo, heir, food scraps, fat, laundry powder, fabric conditioners, toilet paper, chemicals, detergent, household cleaners, dirt, micro-organisms (germs) which can make people ill and damage the environment. Much of the water supplied ends up as wastewater which makes its treatment very important.

Domestic sewage, industrial sewage, and storm sewage are the three types of wastewater or sewage. Domestic sewage, also known as sanitary sewage, transports used water from homes and flats. Water from manufacturing or chemical operations is used in industrial sewage.

4.3 WATER POLLUTION

Water pollution means the contamination of water bodies with pollutants that are potentially harmful to human beings and other living beings. The unpleasant change in water may lead to changes in the colour, taste and/or even the odour of water, rendering it completely unpalatable because of the high level of toxic compounds

present in the water. Water is an essential resource that is required for many basic functions that include water for drinking, bathing, washing and cooking. A high level of contamination of water will, therefore, affect humans immensely and lead to a host of infections and disorders depending upon the level of contamination of water.

4.3.1 Causes of Water Pollution

The causes of water pollution may be classified into two broad categories: point sources and non-point sources.

Point sources

Direct sources of water pollution like effluents from industries that are let into water bodies without being treated are called point sources. The point sources of water pollution are easy to identify and easier to correct when compared with non-point sources of pollution. When large volumes of effluents are discharged into water bodies they cause drastic changes to the quality of the water and result in complete annihilation of aquatic life in extreme cases. Point sources of water pollution include:

- Water pollution caused by oil refinery wastewater discharge outlet
- Noise pollution resulting from jet engines
- Seismic vibrations
- Light pollution by intrusive street lights
- Thermal pollution by industrial processes
- Radio emissions from electrical devices

Non-point sources

These sources of water pollution include sources that are multiple and which pass through various channels before they finally reach the water body. Examples of non-point sources include:

- **Rain water from busy roads:** Rain water that is collected from busy roads carries all the dirt and grime of road along with the existing pathogens. When this water is carried off into lakes it is dangerous for the people drinking water from the lake as well as the lake ecosystem.
- **Water from agricultural fields:** Water from the agricultural fields contains chemicals resulting from pesticides and manures. It affects the aquatic life owing to the toxicity of the chemicals from the pesticides. This is a non-point source of pollution as the water runs from many fields till it finally reaches the water body.
- **Emissions from various factories:** These lead to air pollution whose effects finally reach the water bodies when the emission mixes with water or snow and falls as acid rain. Some important non-point sources of water pollution include:
  - o Excess fertilizers, insecticides and herbicides from agricultural lands
  - o Oil, grease and toxic chemicals carried by the urban runoff

NOTES

- o Sediment from the construction sites, crops and forest lands
- o Salt from irrigation practices
- o Acid drainage from abandoned mines
- o Bacteria and nutrients from pet wastes, livestock and defective septic systems
- o Atmospheric deposition and hydro-modification

4.3.2 Sources of Water Pollution: Domestic, Industrial, Agricultural Soil, and Radioactive Wastes

The main origin or sources of waste water are as follows:

1. **Domestic sewage:** It includes used water from houses and apartments. It is also called sanitary sewage. Domestic sewage is slighly more than 99.9% water by weight. The rest, less than 0.1% contains a wide variety of dissolved and suspended in puri-fies.
2. **Industrial wastewater:** It is the used water from manufacturing or chemical processes. The origin of industrial wastewater is discussed below:

(i) **Textile industry:** Waste water emerges from the textile industry at different stages of processing such as,

- Desizing
- Bleaching
- Mercerising
- Dyeing
- Printing
- Finishing

(ii) **Wood Industry:** Waste water orginates during

- Scouring
- Oiling
- Sizing
- Filling
- Dyeing

The waste water is highly coloured and contains greases, soaps, alkalies, olive oil, starch, soda ash, detergents, chromium salts, etc.

(iii) **Pulp and paper industry:** Waste water is generated during cellulosic raw material preparation, pulping with chemicals, washing, bleaching, chemical recovery, and screening of pulp and paper. The effluent is dark brown, has high content of suspended and dissolved solids (1350 mg/L and 1650 mg/L), high COD (1600 mg/L), etc.

(iv) **Electroplating industry.** Surface cleaning, stripping or pickling and electroplating contribute to waste water stream. The effluents contain NaOH, Na_2CO_3, H_2SO_4, toxic metals, aldehydes, oil, grease, TDS and saccharin etc.

(v) **Leather tanning industry:** Tannery waste water originates during soaking, liming, dehairing, deflushing, deliming, vegetable tanning, bating, pickling, chrome tanning, dying, fat liquoring, etc.

(vi) **Fertilizer and pesticide industries:** Waste water originates from spill over of the final fertilizer products, boiler blow down, cooling processes, etc.

(vii) **Cane sugar industry:** Effluents emerge during spillage from sugarcane juice extraction, clarification, wash waters from filter press, spray pond

overflow, floor washings and spillage from pan boiling. Waste water has high BOD, obnoxious odour, TDS and high carbonaceous matter.

(viii) **Edible oil refinery:** Sources of waste waters are spill-overs, filter washings, neutralisation of excess fatty acids.

(ix) **Oil fields and oil refinery:** Effluents originate during oil pumping, desalting, demulsification and refining operations.

(x) **Soap and detergent industry:** Origins of waste water are spill over of chemicals, floor washings and processing waste. Water contains alkyl benzene sulphonate, builders, borax, surfactants, etc.

(xi) **Food industry:** Food industry encompasses canning, dairies, breweries, distilleries and some pharmaceutical industries. Origins of waste water are spillage, washing of cans, butter, cheese equipments, screening, precipitation, etc.

(xii) **Energy industries:** Waste hot water emerges from thermal and nuclear power plants.

3. **Agricultural runoff:** It is the surface run off from farmland outflow, which comes from the farmland's surplus water. It has complex pollutant compositions including nitrates, ammonium and phosphorus compounds, heavy metals, pesticides and persistent organic pollutants.
4. **Urban run of and storm water:** Urban run off is surface run off of rainwater, landscape irrigation and car washing created by urbanization..

Impervious surface and constructed during land development. During rainstorms and other precifritations events, these surfaces alogwith rooftops carry polluted stormwater to storm drains which finally finds ways to the waterbodies.

4.3.3 Water Pollutants and their Effects

There are various water pollutants which cause water pollution. Its effects are as follows:

- Water bodies in close proximity to metropolitan areas are highly contaminated. This is the result of industrial and commercial businesses dumping rubbish and harmful substances.
- It has a great impact on aquatic life. It has an effect on their metabolism and behaviour, as well as causing illness and death. Dioxin is a toxin that can cause a variety of issues, ranging from infertility to uncontrolled cell proliferation and cancer. Bioaccumulation of this chemical has been found in fish, chicken, and beef. Before reaching the human body, chemicals like these go up the food chain.
- It affects the food chain by disrupting it. For example, cadmium and lead are some toxic substances, enter the food chain through animals (fish when consumed by animals, humans) and disrupt the food chain at higher levels.
- It severely affects health of human beings, causing various diseases, such as hepatitis through faecal matter in water sources.
- It also impacts the ecosystem by modifying and destructing it.

NOTES

4.4 ANALYSIS OF WATER

The analysis of water pollutants water analysis is extremely important as it contains a large number of impurities which are necessary to be checked before the water is used for any specific purpose. Water analysis is usually expressed in milligrams per litre (mg/L) or parts per million (PPM).

$$1 \text{ ppm} = \frac{\text{One part of pollutant impurity}}{10^6 \text{ part of water}}$$

Parts per million (ppm) mean the number of parts of substance per million parts of water.

4.4.1 Objectives of Water Analysis

The objective of water quality monitoring is to obtain quantitative information on the physical, chemical, and biological characteristics of water via statistical sampling.

Control of water pollution necessarily requires quantitative measurements of water pollutions. The main objectives of water analysis are as follows:

1. To assess water quality
2. To provide pure water to public for drinking, domestic and industrial purposes.
3. To choose the most effective treatment method for water purification.
4. To determine the efficiency towards natural purification when sewage and industrial wastes are discharged into water bodies.
5. To trace the origin and extent of pollution and to suggest a possible remedy.
6. To check efficiency, uniformity and consistency of treatment processes.
7. To analyse whether infection by microbial organisms has occured.
8. To find out a particular micro-organism and to suggest preventive measures and effective disinfection procedures.
9. To determine the influence of sewage and wastewater on receiving waters and to protect them from contamination.

4.4.2 Parameters for Water Analysis: Colour, Turbidity, Conductivity, Total Solids, Acidity, Alkalinity, Hardness, Chloride, Sulphate, Fluoride, Silica, Phosphate and Different Forms of Nitrogen

Let us study the different parameters for water analysis in detail.

I. Colour

Pure water is colourless. The presence of pure solids, industrial effluents, metals, tannis, peat, humus, algae, weeds, phytoplanktons, protozoa and suspended water impart colour to the nature water. True colour can be estimated with the naked eye or photoelectrically. The methods for colour analysis are discussed below:

1. Platinum cobalt method

(a) Preparation of stock solution: Dissolve 1 g of $CaC1_2 . 6H_2O$ and 1.245 g of K_2PtC1_6 in minimum quantity of water. Add 100 mL cone. HCl and dilute to 1 L with distilled water. The resulting stock solution has colour value of 500 units.

(b) Colour standards: Prepare standards by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, ..., 7-0 mL of the above solution with distilled water to 50 mL in standard Nessler tubes. The solutions have colour value of 5, 10, 15, 20, 25, 30, ..., 70 respectively. Indicate the colour value on each tube.

Procedure

The method involves the following steps:

1. Centrifuge the water sample to remove suspended matter.
2. Fill 50 mL Nessler tube with clear water sample to a height equal to that of standards.
3. Compare the colour of sample and standards by looking vertically downward through the tubes upon a white or mirrored surface placed at such an angle that light is reflected up. Read the colour value.
4. If the sample shows a colour more than 70 units, it should be diluted with distilled water and the colour estimated is multiplied with dilution factor.

Limitation

Platinum cobalt method is valid only for potable water and not for industrial effluents. It is always necessary to specify pH when colour is measured.

2. Luminescence and colorimetric method

Colour is identified in terms of the dominant wavelength and degree of brightness by luminance. Tristimulus colorimeter with three filters can be used to measure transmittance of water sample.

3. Spectrophotometric method

It is the most reliable method, wherein the absorbance of water is measured between 400-700 nm.

II. Turbidity

Turbidity in water is due to suspended matter like silt, clay organic or inorganic matter, planktons and other micro-organisms. It is an expression of optical property (Tyndall effect) of water which causes light to be scattered and absorbed rather than transmitted. Turbidity of suspension may vary due to different optical properties, refractive indices and particle size of matter. It can be measured by the following methods.

1. By Jackson Candle Turbidimeter

Although standard method but Jackson model permits measurement of turbidity from 25 to 1000 JU.

NOTES

2. By Nephelometer

Lower turbidity values (100 NTU) can be measured by Nephelometer.

Standard turbid suspension: Can be formed by dissalving 1 g of hydrazine sulphate in 100 mL of distilled water. Also dissolve 10 g of hexamethylene tetramine in l00 ml water. Mix 5 mL of each solution in 100 mL volumetric flask and dilute it with distilled water to the mark. This suspension gives a turbidity of 400 NTU.

Procedure

This method involves the following steps:

1. Set the nephelometer at 100 using 40 NTU (10 mL of the above stock solution 100 mL water) standard suspension. Every percent of the scale will be equal to 0-4 NTU.
2. Shake the sample thoroughly. Take the sample in nephelometer tube and read the value on the scale. If the sample has turbidity more than 40 NTU, dilute it, so that turbidity can be read on the same scale.

The turbidity can be calculated using the following method:

Turbidity (NTU) = Nephelometer reading × 0.4 × Dilution factor

III. Conductivity

Conductivity of water varies directly with the temperature and is proportional to its dissolved mineral matter content. Specific conductance can be measured by conductivity meter using dip-type call. The instrument and cell are calibrated with 0.05 m KCl solution (conductivity = 654 μ mhocm^{-1})

Specific conductance, $k = \frac{1}{R} \cdot \frac{A}{I}$

where R is the observed resistance of a column of electrolyte, 1 cm long and cross-section area A cm^2.

Electrical conductivity (EC): EC is a measure of water's capacity to convey electric current. It is directly proportional to area (a) and inversely proportional to length (l).

$EC \propto a/l$ or $EC = \kappa \ a/l$

where κ is proportionality constant called specific conductance.

The instrument consists of an AC salt bridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black. Solubridge (calibrated assembly) gives conductivity of the solution in milli mhos/cm or deci Siemcn/m at 25°C.

A simple Wheatstone bridge circuit is used to measure EC by null method. The bridge consists of known and fixed resistances r_1, r_2, variable standard resistance r_4 and unknown r_3. The variable resistance r_4 is adjusted until a minimum or zero current flows through the AC galvanometer. At equilibrium,

$$\frac{r_1}{r_2} = \frac{r_3}{r_4} \text{ or } r_3 = \frac{r_1}{r_2} \times r_4$$

Since conductivity is reciprocal of resistivity, it is measured with the help of r_3.

Reagents: **Standard KCI solution (0.01 M):** Dissolve 0-7456 g of dry KCl in double distilled water and make up the volume to one litre. It gives EC of 1.413 deci Siemen / m at 25°C.

NOTES

Procedure

The method involves the following steps:

1. Take the water sample in a beaker.
2. Warm up the instrument and calibrate the meter with 0.01 M KCl.
3. Adjust cell constant and temperature of the conductivity meter.
4. Rinse the conductivity cell with distilled water and then with the sample.
5. Connect the conductivity cell to meter and dip in the sample.
6. Adjust the current by rotating the dial so that maximum sensitivity is obtained.
7. Read the conductivity value in *dS/m.* Direct reading may be obtained by digital meters.

Results

Observed values of EC are multiplied by the cell constant and a temperature factor to express results at 25°C.

IV. Total Solids

The term 'solid' refers to the matter that remains as residue upon evaporation. Total solid includes both dissolved solids and suspended solids. Potable waters contain mineral matters in dissolved conditions whereas industrial effluents and sewage contains large amounts of undissolved matter. Following types of solids can be analysed as for given below:

(a) Suspended solids

500 mL of a sample is taken exactly in a volumetric flask and allowed to filter through a dried and weighed Gooch crucible containing an absestos mat . The suspended solids retained in the crucible are washed with distilled water to remove chloride. The crucible is finally dried, cooled in a desiccator and weighed. The increase in the weight of the crucible is equivalent to the suspended impurities present. The total solid contents of 500 mL sample can also be calculated by evaporating it to dryness on a steam bath and drying at about 100-110°C in an oven for about one hour. From this, eliminate the dissolved solids to get the quantity of suspended solids.

(b) Dissolved solids

Filter 500 mL sample in a Gooch crucible to free it from suspended matter and evaporate to about 50 mL. It should be noted that any deposit on the walls of the beaker due to evaporation of water should not touch the flame of the burner. The 50 mL liquid is carefully transferred to a weighed platinum dish with the help of a policeman and wash with distilled water. Evaporate the solution to dryness on steam bath and dry the dish in an oven at about 100-110°C for about an hour. Cool it in a desiccator and weigh.

$$\text{Weight of solids} \times \frac{10^6}{500} = \text{ppm. dissolved solids.}$$

NOTES

Results

Disposal of industrial effluents and sewage contribute suspended solids to the water bodies. The ISI has specified a maximum limit of 30 *mg*/L for suspended solids discharged into river. Solid determination is particularly useful in the analysis of sewage and other waste waters.

V. Acidity

Acidity is a measure of the effects of combination of compounds and conditions in water. It is the power of water to neutralize hydroxyl ions and is expressed in terms of calcium carbonate. Water attains acidity from industrial effluents, acid mine drianage, pickling liquors and from humic acid. It can be measurement.

Titration method

Let us discuss the principle and procedure of measuring of acidity using titration method in detail.

Principle

Acidity of water can be determined by titration with sodium hydroxide solution. The amount of sodium hydroxide required for the sample (pH below 4.5) to reach pH 4.5 (methyl orange and point) is a measure of mineral acidity while the amount of sodium hydroxide to reach pH 8.3 (phenolphthalein end point) is a measure of total acidity. Samples containing acidic wastes (pH below 4.5) correspond to both mineral and CO_2 acidity.

Procedure

Follows the given steps to measure:

(a) Mineral acidity: Take 50 mL or suitable dechlorinated aliquot of the sample in a 250 mL conical flask. Add 2 drops of methyl orange indicator and titrate with 0.02 N NaOH solution till faint orange colour.

(b) Total Acidity at boiling temperature: To 50 mL of the sample, add 5 drops of phenolphthalein indicator. Heat to boil for 2 minutes. Titrate with 0.02 N NaOH solution to pink colour.

Calculation

$$\text{Acidity as } CaCO_3, \text{ mg/L} = \frac{\text{mL titrant (NaOH)} \times 1 \times 1000}{\text{mL sample taken for titration}}$$

Result

Methyl orange acidity shows mineral acidity. In absence of mineral acidity, total acidity is only the CO_2 acidity of the sample.

Total acidity expressed as $CaCO_3$, can also be determined in the following manner. Take 100 mL of a sample in a tall cylinder to decrease the surface of the sample and minimize loss of dissolved carbonic acid during titration. Now add few drops

of phenolphthalein indicator and titrate the solution very rapidly against 0.02 N NaOH with constant stirring until a faint pink colour is obtained.

Calculation

NOTES

$$\text{mL alkali titration} \times \text{normality} \times 0.05 \times \frac{10^6}{\text{Vol. of sample}} = \text{ppm}$$

VI. Alkalinity

Alkalinity of water is due to the presence of carbonate, bicarbonate and hydroxyl ions.

Tetrimetric method

Let us study the principles and reactions involved in the determining alkalinity using titrimetric method in detail.

Principle

Alkalinity is determined by titration with 0.02 N H_2SO_4 using methyl orange and phenolphthalein as indicators.

Reactions

The reactions involved are as follows:

$$2CaCO_3 + H_2SO_4 \longrightarrow CaSO_4 + Ca(HCO_3)_2$$
$$Ca(HCO_3)_2 + H_2SO_4 \longrightarrow CaSO_4 + 2CO_2\uparrow + 2H_2O$$
$$Ca(OH)_2 + H_2SO_4 \longrightarrow CaSO_4 + 2H_2O$$

Reagents

The reagents involved are as follows:

(i) **Sulphuric acid 0.02 N:** Dilute 20 mL of 1N.H_2SO_4 to 1000 mL with distilled water.

(ii) **Sodium carbonate solution:** Dissolve 13.25 g Na_2CO_3 in distilled water to 250 mL.

(iii) **Phenolphthalein indicator solution:** Dissolve 500 mg phenolphthalein in 50 mL alcohol and 50 mL distilled water. Add 0.02 N NaOH solution till light pink colour appears.

Procedure

The method involves the following steps:

1. Take 50 mL of the sample in a 250 mL conical flask.
2. Add 2 drops of phenolphthalein indicator. Titrate the pink colour with 0.02N H_2SO_4 till it becomes colourless. If the sample contains waste waters, then remove the suspended matter by filtration or centrifugation.
3. Determine alkalinity.

Phenolphthalein alkalinity (as $CaCO_3$) mg/L

$$= \frac{\text{mL 0.02 N } H_2SO_4 \text{ for phenolphthalein end point} \times 1 \times 1000}{\text{mL sample taken for titration}}$$

NOTES

Result

Alkalinity measurements are used as the means of evaluating the buffering capacity of waste waters and sludge. It is also significant in determining the suitability of a water for irrigation, in the treatments of natural and waste waters and to calculate the Langelier Saturation Index. Alkalinity provides an idea of the nature of salts present in water. If it is equal to hardness, calcium and magnesium salts are only present in water. If alkalinity is less than hardness, sulphates of calcium and magnesium must be there. Greater alkalinity shows the presence of alkali salts of sodium and potassium in addition to those of calcium and magnesium.

VII. Hardness

Hardness is the soap consuming capacity of water and is expressed as the concentration of Ca^{2+} and Mg^{2+} ions in water as $CaCO_3$ equivalent. Hardness is of two types:

Temporary hardness: It is due to the carbonates of Ca and Mg ions and can be removed by boiling.

Permanent hardness: It is due to the chlorides and sulphates of Ca and Mg ions and cannot be removed by boiling.

It can be determined by the following:

Complexometric titration

Let us study complexometric titration in detail.

Principle

During titration with EDTA (Na_2H_2Y), Ca^{2+} first reacts to form stable CaY^{2-} followed by Mg^{2+} to give MgY^{2-} complex (indicator/wine-red) releasing free indicator (blue). The colour changes from wine-red to blue at the end point.

Reactions

$$Ca^{2+} + H_2Y^{2-} \rightleftharpoons CaY^{2-} + 2H^+$$

$$Mg^{2+} + H_2Y^{2-} \rightleftharpoons MgY^{2-} + 2H^+$$

$$MgD^-_{(red)} + H_2Y^{2-} \rightleftharpoons MgY^{2-} + HD^-_{(blue)} + H^+$$

Procedure for Ca^{2+} and Mg^{2+} hardness

Take 20 to 50 mL sample of hard water in a conical flask. In presence of organic matter in waste waters, add 2 to 4 drops of 30% H_2O_2.

1. Adjust the pH to 8, boil for 15 minutes and cool.
2. Add 5 mL buffer of pH 10 (142 mL conc. NH_3 + 17.5 g NH_4Cl diluted to 250 mL with deionised water) and warm the solution.
3. Add two drops of 1.4% Eriochrome Black T in triethanolamine.

4. Titrate with 0.01 M EDTA solution till the colour changes from, wine-red to blue.

Calculation

$$1 \text{ mL } 0.01 \text{ M DETA} \equiv 1.0 \text{ mg } CaCO_3$$

$$\text{Hardness (mg/L)} = \frac{\text{Vol. of EDTA used} \times 0.01 \text{ M} \times 1000}{\text{mL of sample taken}}$$

This gives total Ca^{2+} and Mg^{2+}.

Procedure for Ca^{2+} hardness

To determine the hardness follow the given steps:

1. To a 100 mL sample add 20% KOH solution to bring the pH to 12 and precipitate Mg^{2+} as $Mg(OH)_2$.
2. Add 5 to 10 drops of calcon carboxylic acid indicator (0.4% in methanol).
3. Titrate with 0.05 M EDTA under magnetic stirring till the colour changes from wine-red to pale blue.

Alternatively, add drops of mureoxide indicator (0.1 g stirred with 2.5 mL deionised water and filtered). Titrate with 0.05 M EDTA solution till the colour changes from orange to violet.

Calculation

1 mL of 0.01 M EDTA ≡ 0.4008 mg Ca.

VIII. Chloride

Chloride in drinking water is harmless if present below 250 ppm but its higher content harms metallic pipes and crops. It can be determined by the following method:

Determination by Mohr's method

Chloride is determined by titration with $AgNO_3$ solution using K_2CrO_4 as an indicator. The end point is indicated by the appearance of reddish tinge. The method is valid for 0.16 to 10 mg Cl^- and electrical conductivity of water is greater than 1 dS/m at 25°C.

Procedure

The method involves the following steps:

1. Take 100 mL sample in 250 ml, conical flask. Adjust pH from 7 to 10 with H_2SO_4 or NaOH (0.5 g $Na_2B_4O_7$ will keep the pH at 9).
2. Add 1 mL of 5% K_2CrO_4 indicator with stirring.
3. Titrate with 0.0282 N $AgNO_3$ solution (282 mL of 0.1 N $AgNO_3$ diluted to 1 litre) to a reddish tinge.

$$Cl^- + Ag^+ \rightleftharpoons AgCl; \quad 2Ag + CrO_4^{2-} \longrightarrow Ag_2CrO_4$$

NOTES

NOTES

Calculations

1 mL of 0.0282 N $AgNO_3$ = 1 mg Cl^-

$$\text{or } Cl^- \text{ (mg/L)} = \frac{\text{Normality } AgNO_3 \times \text{Vol. o } AgNO_3 \times \text{Eq. wt. of } Cl^-}{\text{mL o sample taken}}$$

Potentiometric titration is performed for smaller quantities of chloride with $AgNO_3$ using glass and Ag—AgCl electrode system.

IX. Sulphate

Sulphate usually occurs in natural waters. Mine drainage wastes also contain high content of sulphate by virtue of pyrite oxidation. The presence of Na_2SO_4 and $MgSO_4$ in drinking water beyond the prescribed limits may cause cathartic action. It can be determined by the following methods:

Gravimetric Method: In gravimetric procedure, sulphate is precipitated as $BaSO_4$ in acidic (HCl) medium using $BaC1_2$ solution. The precipitate of $BaSO_4$ is digested, filtered and washed with hot water to remove Cl^- ions, ignited and weighed.

Volumetric Method: 100 mL of the sample water is taken in a conical flask and add 10 mL of benzidine hydrochloride solution (solution of benzidine in dilute HCl containing 4 g. of the diamine base per litre of the solution). The precipitate of benzidine sulphate is filtered and washed free of acid with minimum amount of distilled water. The precipitate and filter paper are taken in a conical flask and 50 mL of distilled water are added to it. Now few drops of phenolphthalein are also added.The conical flask is well shaken to dissolve the precipitate and the solution so obtained is titrated against *N/7* NaOH until pink colour is obtained at the end point.

Calculation

Sulphate (as Na_2SO_4) ppm = No. of mL of N/7 NaOH × 100. Na_2SO_4 ppm can be converted into $CaCO_3$ ppm by multiplying with a factor of 0.705.

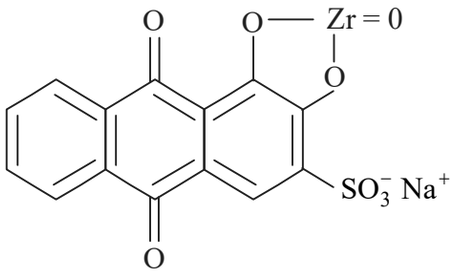
X. Fluoride

Fluoride occurs is all natural water supplies and in chemical wastes from industries. Fluorides if present in small quantities upto 1 ppm, are generally considered as beneficial. Excessive fluorides in drinking water may cause mattling of teeth or dental fluorosis, which results in decolouration of enamel and chipping of teeth in children in severe cases. Bone fluorosis or crippling effects are also seen with excess of fluoride concentration in drinking waters. Determination of fluoride in water can be done by spectrophotometric and ion- selective electrode methods discussed below.

Spectrophotometric method

This method involves the following methods:

(i) **Alizarin-S visual method:** Fluoride reacts with Zr Alizarin-S lake to form colourless ZrF_6^{2-} and the dye. the colour of the dye lake becomes progressively weak with increase in amount of F^-.



Zr-Alizarin Red-S Lake

To a 100 mL sample add 1 drop of $NaAsO_2$ solution (5g/L) to remove residual Cl, if any. Add 5 mL acid-zirconyl-alizarin reagent (300 mg $ZrOCl_2.8H_2O$/ 50 mL + 70 mg alizarin red S/50 mL + 800 mL of 1•5 N HCl - 1.2 N H_2SO_4 made up to 1 litre). Mix thoroughly and compare the samples and standards after 1 hour.

(ii) **Spadns method**

Spadns method is preferred to the Alizarin visual method as the latter takes 1 hour for colour development. (Spadns is Sodium 2-(para sulphophenylazo)-1, 8-dihydroxy-3, 6 naphthalene disulphonate). The reaction rate between P- and ZrO^{2+} is influenced greatly by the acidity of the reaction mixture. By increasing the proportion of acid in the reagent, the reaction can be made practically instantaneous.

Procedure

The method involves the following steps:

1. Prepare a series of standard solutions of F^-, i.e., 0.5, 1.0 and 2.0 mg/L.
2. Add 1 drop of $NaAsO_2$ solution (0.5%) to remove any residual chlorine.
3. Dilute sample to 50 mL. Add 5 mL of SPADNS (1•9 g/L) and 5 mL of zirconyl acid reagent (0•26 g $ZrOCl_2.8H_2O$ and 700 mL of 50% cone, HCl/L).
4. Mix well and read absorbance at 570 nm against standard immediately. The concentration of fluorine from a standard curve.

(ii) Ion-selective electrode method

This is the most convenient method for the estimation of F^-, down to 10^-5 M (0•2 mg/L). It is based on potentiometric measurements with a membrane electrode consisting of a single crystal of europium doped lanthanum fiuoride LaF_3. The purpose of Eu doping is to improve electrical conductivity. The membrane is cut as a 1-mm thick disc, a few mm in diameter. The disc is sealed into the end of a rigid plastic tube, filled with an equimolar solution of KCl and NaF, into which dips a AgCl electrode. A reference electrode (saturated calomel electrode) is inserted into the test solution along with the fluoride electrode. The potential difference is measured.

Cell Ag/AgCl, Cl^- (0.3 M), $\overline{F}$ (0•001 M) | LaF_3 | test solution/reference electrode

$$E_{meas} = \text{Const} + \frac{RT}{nF} \log ar\, F^- = 0.058 \log [F^-] + \text{constant}$$

NOTES

Plastic beakers and flasks (polyethylene, teflon) should be used for storing F^- solution and for all measurements.

Procedure

The method involves the following steps:

1. Prepare a series of standards equivalent to 0.5 to 1.0 and 2.0 mg/L of fluoride.
2. Take 50 mL sample, add 50 mL buffer solutions of high-ionic strength (pH 5.0 to 5.5) (glacial acetic acid + NaCl + cyclohexylene diamine tetracetic acid, CDTA + NaOH adjusted to pH 5.0 to 5.5).
3. Transfer each standard and sample to a series of 150 mL beakers. Stir test solution on a magnetic stirrer. Measure the developed potential.

XI. Silica

Silica in water is not a pollutant, but its higher concentration in water is undesirable. It can be removed by the use of strongly basic anion-exchange resin in the deionization process or by distillations. The silica content in natural water is usually 1-30 mg/L. Bracksh water and brines way contain as high as 1000 mg/L of silica the gravimetric method of determination is useful for 20 mg/L as more of SiO_2 and spectrophotometric method for concentration 0.4–0.25 mg/L of SiO_2. It can be determined by the following method.

Gravimetric method

Silicates and dissolved SiO_2 are decomposed by HCl giving silicic acids during evaporation. Ignition completes dehydration of SiO_2 which is weighed and then volatilised as SiF_4, leaving impurities as non-volatile residue. The residue is weighed and the difference gives SiO_2 lost on volatilisation.

$$Na_2SiO_3\ (SiO_2) + 2HC1 \longrightarrow 2NaCl + H_2SiO_3$$

$$H_2SiO_3 \longrightarrow SiO + H_2O$$

$$SiO_3 + 4HF + 2H_2SO_4 \longrightarrow SiF_4\uparrow + 2H_2O + 2H_2SO_4$$

Procedure

The method involves the following steps:

1. Take a clear sample (10 mg SiO_2) in a 200 mL platinum dish. Add 5 mL 6N HCl and evaporate repeatedly with addition of HCl to dryness on a water bath.
2. Bake the residue on a hot plate for half an hour.
3. Leach with 5 mL 6N HCl, warm and add 50 mL hot distilled water. While hot, filter through an ashless filter paper. Wash the dish and residue with hot 0.2 N HCl and then with small volume of distilled water till the filtrate is chloride free.

4. Evaporate the filtrate and washings from the above step to dryness in the original platinum dish and repeat steps 2 and 3 given above.
5. Transfer the two filter papers and residues thus obtained to a covered and weighed platinum crucible, dry at 110°C and finally ignite at 1200°C to constant weight.
6. Moisten the residue in the crucible with distilled water. Add 4 drops of 18 N H_2SO_4 and then 10 mL HF. Slowly evaporate to dryness over a hot plate in a hood. Ignite the crucible al 1200°C to constant weight.
7. Record the weight of SiO_2 as the difference in these two weight from steps 5 and 6.

Principle

Ammonium molybdate at pH 1.2 reacts with SiO_2 and any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid, $H_3PMo_{12}O_{40}$ but not the molybdosilicic acid, $H_4SiMu_{12}O_{40}$. The resulting yellow molybdosilicic acid is measured at 650 nm.

Procedure

On the basis of the given principle, follow the given steps:

1. Place 50 mL sample in a 100 mL platinum dish and 200 mg $NaHCO_3$ and digest on a steam bath for 1 hour. Cool and add slowly with stirring 2.5 mL H_2SO_4.

   (Note: The digestion step is necessary to convert any molybdate unreactive silica to the reactive form). Make the volume to 50 mL and transfer to a 100 mL separatory funnel.
2. To 50 mL treated sample, add quickly 1 mL of 6 N HCI and 2 mL ammonium molybdate. Shake and allow to stand for 10 minutes.
3. Add 1.5 mL oxalic acid (10 g in 100 mL distilled water) and shake vigorously. Measure the absorbance at 650 nm against a reagent blank.

XII. Phosphate

Industrial effluents, domestic sewage and agricultural run offs are the major contributors of phosphates in water. It can be determined by following methods:

Spectrophotometric method

Let us study the method in detail.

Principle

Orthophosphates form heteropoly acid when they react with ammonium molybdate and potassium antimony tartrate in acid medium. The phosphomolybdic acid reduces to molybdenum blue by ascorbic acid .

Reagents

Some of the following reagents are as follows:

- **Standard phosphate solutions:** Dissolve 2.194 g of anhydrous potassium hydrogen phosphates in deionised water and make up the volume to 500

NOTES

mL. Take 10 mL of this solution and add deionised water to make 1 L of stock solution containing 1 mg P/L. Prepare standard phosphorus solutions of various strengths from 0•0 to 1.1 mg *P/L* at intervals of 0•l mg *P/L* by diluting the stock solution with distillcd water.

- **Reagent *A:*** Take 1 g of ammonium molybdate and 0.02 g of potassium untimony turtratc in 1000 mL volumetric flask. Add 16 mL of concentrated H_2SO_4 slowly. Dilute with distilled water to the mark.
- **Reagent B:** Weight 0.88 g of ascorbic acid and dissolve in 1 L of reagent A.

Procedure

The method involves the following steps:

1. Take 25 mL sample in an Erlenmeyr flask and evaporate to dryness. Cool and dissolve the residue in 1 mL of 70% $HClO_4$.
2. Heat the flask gently so that the content becomes colourless. Cool and add 10 mL distilled water and two drops of 1% phenolphthalein indicator.
3. Titrate against 1 N NaOH solution until pink colour appears. Make up the volume to 25 mL with distilled water.
4. Transfer this solution into 50 mL volumetric flask and add 10 mL of reagent B.
5. Make the volume to 50 mL with water till the blue colour develops.
6. Record the absorbance on spectrophotometer at 660 nm.
7. Run simultaneously a distilled water blank in the same manner.
8. Process the standard phosphorus solutions of various strengths in a similar way.
9. Plot a curve between absorbance and concentration of standard phosphorus solution.
10. Deduce the phosphrus content of the sample by comparing its absorbance with standard curve.

Calculation

$$\text{P (mg / L)} = \frac{\text{mg } P \text{ in 50 mL}}{\text{Volume of sample}} \times 1000$$

XIII. Different forms of nitrogen

Nitrogen occurs in water in small amounts and in bound form, it is found as NH_3, NO_3^-, NO_2^- and organic nitrogen. Let us study them in detail.

(i) Ammonia: Ammonia is present in surface water, ground water and domestic sewage. In water bodies, it is produced naturally by the reduction of nitrates under anaerobic conditions. Titration procedures are valid when the NH_3 level exceeds 5 ppm. Distillation is necessary when the sample is coloured and when the titrimetric method is followed.

Spectrophotometric Nessler's method: The method is useful for ammonia nitrogen upto 5 ppm. It is based on the reaction between NH_3 and HgI_4^{2-} tetra-iodo mercury (II) anion in alkaline solution.

$$NH_3 + 2\,HgI_4^{2-} + 3OH^- \longrightarrow NH_2Hg_2IO + 7I^- + 2H_2O$$

Tho orango-brown reaction product tends to precipitate at higher concentration, while at lower concentration it is measured at 420 nm spectrophotometrically.

Procedure 1

It involves the following steps:

1. To a 100 mL sample, add a little NaOH to neutralise the acid used for storage and then add l mL 10% $ZnSO_4$. $7H_2O$ followed by 1 mL of 10% NaOH. Stir and filter Ca, Fe, Mg, S^{2-} are precipitated.
2. Collect the colourless middle fraction, add a drop of 50% EDTA (disodium salt), mix well and add 2 mL of Nessler's reagent [70 g KI+ 160 g HgI_2 + 160 g NaOH (ice-cooled) diluted to 1 litre]. Shake well.
3. Measure the resulting yellow colour at 420 nm.

Procedure 2

It involves the following steps:

1. When the sample is coloured, distil a 500 mL sample with dil. NaOH and collect the distillate in an Erlenmeyer flask containing 200 mL of 0.1 N H_2SO_4.
2. Make up the volume of distillate to 250 mL in a volumetric flask.
3. Take 5–10 mL aliquot, neutralise with 0.1 N NaOH to pH 4 and add 2 mL Nessler's reagent. Proceed as above for measurement.

Procedure 3

It involves the following steps:

1. Distil as above and collect only 100 mL of the distillate in an Erlenmeyer flask.
2. Titrate with 0.02 N H2SO4, using a mixed indicator (200 mg methyl red in 100 mL 95% ethyl or isopropyl alcohol + 100 mL methylene blue in 50 mL 95% ethylalcohol), until the indicator turns a pale lanvender.
3. Carry a blank through all the steps.

2. Nitrate and nitrite: Nitrate and nitrite are usually monitored regularly in water supplies as they are deemed to be potentially hazardous to health if their maximum admissible concentrations of 50 mg/Land 0.1 mg/L, respectively, are exceeded. NO_3^- is particularly dangerous to infants less than six months old, causing a child disease, methemoglobinaemia. A limit of 10 mg/L for NO_3 has been imposed on drinking water.

Nitrates generally occur in traces in surface water and ground water. Nitrites are an intermediate product, both in the oxidation of NH_3 to NO_2 and in the

NOTES

reduction of NO_3, which occurs in waste-water treatment plants, water-distribution systems and natural waters.

Spectrophotometric method for total nitrate and nitrite: Nitrates and nitrites are reduced to NH_3 by Devarda's alloy (50 Cu, 45 Al, 5 Zn) in strongly alkaline solutions. The NH_3 is distilled into excess standard acid and finally estimated spectrophotometrically.

$$3NO_3^- + 8I + 5OH^- + 2H_2O \longrightarrow 8AO_2^- + 3NH_3$$

Procedure

The method involves the following steps:

1. Take 500 mL sample in NH_3 distillation apparatus. Add 50 mL of 10% NaOH and evaporate to about 200 mL.
2. Cool the solution, add 3 g Devarda's alloy (20 mesh) and then 30 mL of 10% NaOH and immediately connect the flask with a vertical condenser whose outlet dips into a receiver containing 200 mL of 0.2 N H_2SO_4.
3. Disconnect the receiver. Make up the volume of the solution in the receiver to 250 mL. Take 5 to 10 mL aliquot in a 50 mL volumetric flask and neutralise to pH 4.5.
4. Add 2 mL Neasler's reagent and estimate the absorbance at 424 nm as described under ammonia.
5. This spectrophotometric method is valid for NO_3^- concentrations greater than 0.5 ppm.

Titrimetric method: It may be followed for NO^{3-} content exceeding 5 ppm. Here the distillate can be directly back titrated with standard alkali (0.2 N NaOH) using methyl red as indicator.

$$1 \text{ mL N } H_2SO_4 \equiv 0.06201 \text{ g } NO_3^-$$

3. Nitrite: It includes the method which is based on diazotisation reaction. A reddish purple azo dye is produced at pH 2.0 to 2.5 by the coupling of diazotised sulphanilamide with N-(l-naphthyl) ethylenediamine dihydrochloride.

Procedure

It involves the following steps:

1. Take a 40 mL sample in a volumetric flask and adjust pH to 7.0.
2. Add 2 mL of sulphanilamide solution (50 g in 500 mL of 1.2 N HCl), shake and allow to stand for 10 minutes.
3. Add 2 mL of N-(1-naphthyl) ethylene diamine dihydrochloride (0•83 g in 200 mL warm water, cooled, filtered and diluted to 250 mL with glacial acetic acid), dilute to 50 mL and mix thoroughly.
4. Measure the resulting purple azo dye at **543 nm** within two hours, also against standards covering the range of NO_2^- from 1 to 25 μg/L.

It can includes the following methods:

4. Total Organic Nitrogen (TON)

Titrimetric method

Organic nitrogen occurs in the form of amino acids, urea and nucleic acid.

NOTES

The materials used in the method are as follows:

1. Micro-Kjeldahl distillation assembly.
2. **Digestion mixture:** Dissolve 16.25 g of K_2SO_4 in 200 mL of distilled water. Add 0.4 g of mercuric oxide and 25 mL of concontrated H_2SO_4 slowly. Make up the volume to 250 mL with distilled water.
3. **Hypo solution:** Dissolve 50 g of NaOH in 200 mL distilled water and add 10 g of sodium thiosulphate. Make up the volume to 250 mL with distilled water.
4. **Mixed indicator:** Mix 0.1% methyl red solution and 0.5% bromocresol green in 1 : 2 ratio in methanol.

Procedure

The method involves the following:

- Take 200 mL of the sample in a dish and evaporate to dryness .
- Add 4 mL of digestion mixture to the residue and dissolve it in 20 mL, of distilled water.
- Heat the solution for 15 minutes. Cool and transfer the digest to micro-Kjeldahl distillation assembly. Add 5 mL of hypo solution.
- Take 5 mL of 1% boric acid solution containing 2 drops of mixed indicatorr in a conical flask.
- Place the flask below the condenser so that the tip of outlet of the condenser is dropped in contents of the flask.
- Heat the Kjeldahl flask. Continue distillation for 10 minutes. Remove the conical flask having distillate.
- Titrate the distillate against 0.01 N HCl till the colour changes from blue to pink.
- Run a blank using distilled water in a similar manner.

Calculation

$$\text{TON (mg/L)} = \frac{(S - B)\ 0.01\ N \times 1000 \times \text{At. wt. of } N_2(14)}{\text{Volume of sample}}$$

where S = Volume of HCl used against sample.
B = Volume of HCl used against blank.

5. Dissolved organic nitrogen (DON): Take the sample and filter through millipore filter paper, Employ the above procedure of TON. Express the result of dissolved organic nitrogen in mg/L.

6. Particulate organic nitrogen (POM): Determine the dissolved organic nitrogen and total organic nitrogen as describe above and calculate particulate organic nitrogen.

$$\text{PON (mg/L)} = \text{TON} - \text{DON}$$

NOTES

Check Your Progress

1. What is wastewater?
2. Name the major sources of wastewater.
3. When should a composite sample be taken?
4. What does Jackson model permit?
5. Which factors affect conductivity of water?
6. What does the term 'solid' refer?
7. What does acidity measure?

4.5 HEAVY METAL POLLUTION

Heavy metals are defined as metallic elements that have a relatively high density compared to water. With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure. In recent years, there has been an increasing ecological and global public health concerns associated with environmental contamination by these metals. Also, human exposure to these heavy metals have risen exponentially drive to their use in several industrial, agricultural, domestic and technological applications. Sources of heavy metals in the environment include geogemic, industrial, agrcultural, pharmaceutical, domestic effluents, and atmospheric sources. Heavy metal pollution is very prominent in areas such as mining, foundries and smeltens, and after metal based industrial operations. Traces of heavy metals such as Hg, Cd, Pb, Ag, Co, Cr, Mn, Fe and Se have been identified as cumulative poisons causing deleterious effects in living organisms. The toxicity due to heavy metals is mainly determined by:

- Its solubility, stability, biological reactivity, physical form at the site of action and the presence or absence of a homestatic mechanism.
- The delivery of the metal to the cell to attain a critical concentration at the active site.
- The cellular biochemical defence mechanisms.

The actual toxic action of each metal is different but most of them bind to the metabolically active groups such as carboxyl, phosphoryl, amino, imino, phenolic, sulphydryl or imadazole group.

The various factors responsible for heavy metal toxicity are:

- Solubility, stability and reactivity of heavy metal or its complexes
- Oxidation state and electrochemical character of the metal
- The rate of transport of heavy metal complex in blood, its distribution and retention in the body tissues
- The ability of the heavy metal to chelate with various ligands in the body tissues and reactivity of the chelate thus formed

- The efficiency of the enzymatic and homeostatic mechanism which controls the absorption, distribution, retention and excretion of the heavy metal ions or complexes

Various reasons for the adverse effects of metal toxicity in biological systems are as follows:

- Interaction of the metal with protein leading to denaturation
- Interaction with DNA causing mutation
- Effect on cell membranes and regulatory enzymes

The adverse effects in mammals may manifest in the following disorders:

- Pathological changes
- Retardation of growth
- Symptoms of chronic disease
- Decrease in longivity
- Deterimental changes in the reproductive cycle causing mortility in the offspring

4.5.1 Public Health Significance of Heavy Metals: Cadmium, Chromium, Copper, Lead, Zinc, Manganese, Mercury and Arsenic

Due to the high degree to toxicity, arsenic, cadmium, chromium, lead and mercury are the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower level of exposure. They are also clasified as human carcinogens (known or probole) according to U.S. Environmental Pratection Agency. These heavy metals and their significance in public health are discussed in below sections.

I. Cadmium

Let us study cadmium in detail.

Occurrence

Cadmium (Cd), a toxic heavy metal, occurs in nature in association with zinc minerals (1 : 200 in ZnS). The maximum permissible concentration of Cd in water is 5 mg g^{-1} as per the WHO guidelines. Normal Cd levels are less than 1% μg in blood. 'Tolerable daily intake of Cd is 57 to 72 μg per day. Normal Cd content in rice is about 29 ppb.

Industrial uses und pollution sources

Cadmium is used in the manufacture of alloys, paint-pigments, plastics, Ni-Cd batteries and in electroplating. Cadmium stearates are used as stabilizers in the production of PVC plastics. CdO is used in glass manufacture, ceramic glazes and ceramic alloys. Cadmium selenide is used in photoelectric cells, rectifiers and phosphors.

Over one million kg of Cd are released into the air every year from smelters and factories processing Cd which accounts for 45% of total Cd pollution. About

52% of Cd pollution occurs from the incineration or disposal of Cd bearing products like automobile tyres, motor oils, coal and plastics.

Toxic effects of cadmium on man

NOTES

Some of the toxic effects on human being are as follows:

- The outbreak of Cd-poisoning occurred in Toyama city of Japan in the form of itai-itai or ouch-ouch disease. The bones of these victims became fragile, brittle and shattered accompanied by shrieks of tormenting pain.
- Cadmium acts as a potent inhibitor of several enzymes like adenosinc triphosphate, amylase, peptidase, carbonic anhydraso, glutamic oxaloacetic transminase, alcohol dehydrogenase, cholinesterase, tryptophan oxygenase, leucine amino peoptidase and aldolase.
- Cadmium has a strong affinity for sulphur containing ligands such as SCH_3 and SH in methionine and cysteine amino acids. It has also got affinity for other ligands like hydroxyl, carboxyl, phosphatyl and cysteinyl side chains of proteins, purines and porphyrin. It can disrupt path ways of oxidative phosphorylation.
- Cadmium causes strong inhibitory effect on metalloenzymes such as alkaline phosphatase, carboxy peptidase, thermolysin (Zn-containing enzymes) and ceruplasmill (Cu-containing enzyme), The specificity of the enzyme is altered when Zn^{2+} or Cu^{2+} ions are replaced by Cd^{2+}.
- Cadmium toxicity causes anaemia, hypertension, adrenal dysfunction, bone marrow disorders, cancer, and damage to kidneys lungs and liver.
- Cadmium is highly toxic becuase of the absence of homeostatic control for Cd in human body. 1t is bound by body proteins, metallothionein, present in kidneys arnd gradually accumulates with age causing metabolic disorders.
- Cadmium exposure in man results in aminoaciduria (urinary excretion of ammino acids), glucosuria (excretion of blood sugar in the urine), hypercalciuria (urinary excretion of excessive Ca), osteoporosis (decalcification of the skeleton) and proteinuria (urinary excretion of proteins).

II. Chromium

Let us study chromium in detail.

Occurrence

Chromium is distributed in the earth crust at about 200 ppm levels and in sea water at 3 ppb. In nature, Cr occur as chromium iron are ($FeO \cdot Cr_2O_3$). The Cr concentration in human's blood is found to be 0.5 to 5mgL^{-1}, in urine 5 to 10 mgL^{-1} and in hair over 500 ng g^{-1}.

Uses and pollution sources

Chromium is an ingredient of stainless steel. Cr_2O_3 is used in chrome plating, copper stripping, photography and as a corrosion inhibitor. Chromium sulphate is used as a mordant in textile manufacture, in leather tanning, paints, varnishes, inks and glazes for porcelain. Wastes from industrial units contain soluble chromate salts which is a source of water pollution.

Functions of Chromium

Some of the functions of chromium on health are as follows:

- Chromium, in its trivalent form, is essential to man since it plays a vital role in insulin metabolismn as the glucose tolerance factor. Supplementation of chromium helps to improve glucose tolerance in diabetics, older people and malnourished. Most mammalian species tolerate about 100 times their normal body burden of Cr(III) without experiencing any adverse effect.
- Chromium deficiency is characterised by impaired growth and by disturbances in glucose, lipid and protein metabolism.
- Orally administered Cr(III) is poorly absorbed (1% only) than Cr(VI). Absorbed anionic Cr(VI) readily passes through the membrane of red blood cells and gets bound to globin fraction of haemoglobin. Cationic Cr(III) is unable to pass through the membrane. It combines with β-globulin fraction of the plasma protein and transported to the tissues bound to siderophilin.
- The chromium entering the tissues is distributed among the subcellular fractions and is mobilized from the body stores to glucose administration.

Toxic effects of Cr(III)

Chronic exposure to chromate dust has been correlated with increased incidence of lung cancer. Oral administration of excessive levels (50 ppm) has been associated with liver, kidney damage and depression.

Toxic effects of Cr(VI)

Cr (VI) is 100 times more toxic than Cr (III). Some of its harmful effects are as follows:

- Exposure to Cr (VI) causes allegric skin irritations, dermatitis, conjuctiva, gastro-intestinal ulcers and irritation to mucous membranes.
- Cr(VI), a teratogen, is also mutagenic and carcinogenic including bronchogonic cancer.
- Higher level of Cr(VI) causes chrome holes, that is, penetrating ulcers occur around finger nails, joints, eyelids and on forearms. Lesions on the nasal mucosa may lead to performation of the septum. Alkali biochromats can be absorbed through skin lesions to cause renal damage.

III. Copper

Let us study copper in detail.

Occurrence

Copper, an essential chalcophile, is ubiquitous in earth crust as sulphide deposits along with Pb, Cd and Zn. Soluble copper levels in contaminated water range from 0.5 to 2 μgL^{-1}.

Uses and pollution sources

Copper is also used in the manufacture of alloys paints, ceramics and pesticides. Contamination of air with copper occurs near industrial smelters, fertilizer industry,

and iron and steel industry. Water pollution by copper results from the discharge of mine tailings, flyash, disposal of municipal and industrial wastes.

Functions Effects of Copper

NOTES

A daily dietary intake of 2 to 3 mg of Cu is recommended for human adults. Copper absorption and retention depend on factors such as the chemical form in which the metal is ingested, dietary levels of other minerals and the acidity of the intestinal contents in the absorptive area. Some of the effects of copper on health are as follows:

- Copper is necessary for the normal biological activities of amine oxidase and tyrosinase enzymes. The former enzyme is involved in the formation of elastin and collagen. Elastin is the major protein constituent of the walls of large blood vessels while collagen is the proteinaceous component of tendons and bones.
- The Cu-containing enzyme, tyrosinase, is required for the catalytic conversion of tyrosine to melanin, the pigment located beneath the skin protecting it from radiation injuries. Tissues lacking tyrosinase cannot produce melanin and thus would extremely be sensitive to sunlight and probably be prone to early death.
- Defects in bone formation, pigmentation, reproduction, myelination of the spinal cord, cardiac function, connective tissue formation, defects in growth and hematopoiesis were found to be the manifestations of copper deficiency.
- Cytochrome oxidase, ascorbic acid oxidase, laccase, tyrosinase, urinase, amine oxidase, aminolevulinic acid dehydrase and dopamine hydroxylase are all copper enzymes.
- The ingestion of 25-75 mg of copper causes gastro-intestinal disturbances. The intake of large quantities of copper salts may result in hemolysis, hepatotoxic and nephrotoxic effects. Generally, copper toxicity would be aggravated by low dietary zinc or molybdenum.
- Copper deficiency results in Menke's disease whereas excessive accumulation of Cu causes Wilson's disease, thalassemia (Mediterranian anaema), hemachromatosis, cirrhosis, atrophy of liver, tuberculosis and carcinoma.

Toxic effects of copper

Some of its harmful effects are as follows:

- Inhalation of air borne copper causes irritation of the respiratory tract and metal fume fever.
- Workers involved in the spraying of vineyards with Bordeaux mixture (a fungicidal preparation containing about 2% of copper sulphate) develop a respiratory disorder known as vineyard sprayers lung disease. This condition is characterised by the development of interstitial plumonary lesions and nodular fibro-hyaline scars.

IV. Lead

Let us study lead in detail.

Occurrence

NOTES

Lead (Pb) occurs in nature chiefly in lead minerals galena, anglesite and cerussite. The major source of air-borne lead is the combustion of leaded petrol/gasoline. Lead is added in the form of tetra alkyl lead together with scavengers, 1, 2-dichloro (or dibromo) ethane. Busy streets contain 1000 to 4000 mg kg^{-1} of Pb.

Industrial uses and pollution sources

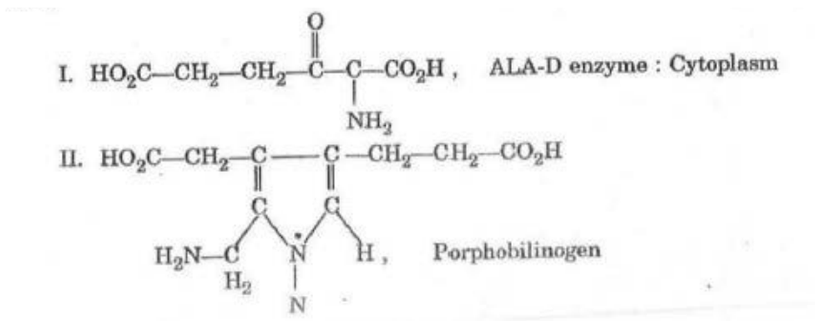
Lead is used in the manufacture of pipes, pottery, alloys, paints, pigments, varnishes, pesticides, etc. Main sources of Pb pollution are mining, smelting of Pb ores, emission from automobile exhausts, effluents from storage battery industries, etc.

Toxic effects of lead

Some of its harmful effects are as follows:

- Lead intake by a city dweller is from food (200 μg per day), air and water (10 μg per day each).
- Lead interferes with heme synthesis which leads to hematological damage. It is implicated in the metabolism of delta-amino levulinic acid (ALA) to porphobilinogen (PBG) and in the formation of heme from iron and protoporphyrin (PP). Both of these steps are mediated by free sulphydryl dependent enzymes, viz. ALA-dehydrase (ALA-D) and heme synthetase.

  An important phase of heme synthesis is the conversion of ALA-D to PBG. Lead inhibits ALA-D enzyme (I) so that it cannot form PBG(II). The overall effect is the disruption of the synthesis of haemoglobin and other respiratory pigments, such as cytochromes, which require heme. Finally, Pb does not permit utilisation of O_2 and glucose for life sustaining energy production.



- Lead can even disturb the structure-function relationships of DNA molecules. It can combine with specific biochemical residues like sulphydryl, carboxyl and imidazol groups.
- Lead inhibits acetylcholinesterase, ATP, cytochrome oxidase, carbonic anhydrase and alkaline phosphate.
- Elevated lead levels (< 0•8 ppm) in the blood cause anaemia, facial pallor, gradual paralysis of muscles, atrophy, insomnia, tremor, dizziness, arthralgia, arteriosclerosis and cardiorenal manifestation.

NOTES

- Chronic lead poisoning results in vertigo, dyspepsia, reticulocystosis, porphyrinuria, sterility, anorexia, vomiting and brain damage.
- Due to chemical analogy of Pb^{2+} with Ca^{2+}, bones act as repositories for Pb. This Pb may be remobilised along with phosphates from the bones which exert toxic effects to soft tissues.

V. Zinc

Occurrence. Zinc is an essential metal occuring as zinc blende (ZnS), zincite (ZnO), willemite (Zn_2SiO_4) and smithsonite ($ZnCO_3$). It occurs as 0.004% in earth crust and 20 ppb in oceans. Zinc content in milk lies between 3 to 5 $\mu g\ mL^{-1}$, in wheat germ and bran from 40 to 120 ppm. Oysters, the richest source of zinc contains 1000 ppm of zinc. Human body contains 300 mg zinc distributed in muscles (65%), bones (20%), plasma (6%), RBCs (2.8%) and liver (53%).

Uses and pollution sources

Zinc is used in dry batteries, pigments, protective coatings on iron, printing processes and construction materials. Smelting of ores and agricultural use of pesticides like zineb and ziram are the pollution sources of zinc. Zinc salts being astringent and antiseptic have limited use in medicine.

Function of zinc

Some of the function are as follows:

- Zinc is present in the body in metalloenzymes such as carbonic anhydrase, alkaline phosphates and dehydrogenase or as co-factors in other enymes (e.g., arginase and histamine diaminase), taking part in the synthesis of DNA, proteins and insulin.
- Several zinc enzymes act as catalysts for RNA and DNA metabolism, that is, for thymidine kinase and polymerase.
- About 80 enzymes including aldolase, alcohol dehydrogenase, carboxy peptidase and lactic acid dehydrogenase are dependent on zinc.
- Zinc is essential for the normal functioning of cell including protein synthesis, carbohydrate metabolism, cell division and growth. It is also required for the synthesis of tryptophan which is a precursor to auxin biosynthesis.
- Zinc has a wound healing capacity. It is present in plasma, bones, hair, nails, blood and also in eyes, liver, kidney, musde, pancreas and heart.
- Recommended daily dietary allowance of zinc is 15 mg for adults.
- Zinc deficiency syndrome manifests itself by retardation of growth, anorexia, lesions of skin and appendages, impaired development and function of reproductive organs.

Toxic effects of zinc

Some of its harmful effects are as follows:

- Toxicity of zinc may be due to other associated metals like Cd, Pb, Sb and As.

- Inhalation of Zn dust and ZnO fumes causes metal fume fever, depression, cough, vomiting, headache and salivation.
- Gastrointestinal symptoms appear within 12 hours following ingestion of 220 to 440 mg of $ZnSO_4$.
- Ingestion of $ZnCl_2$ results in acute renal failure. But the emetic action of zinc may be a protective mechanism.
- Electrolytic imbalance, dehydration, lethargy, dizziness, pneumonitis and uncoordination may occur due to excessive zinc.

VI. Manganese

Let us study manganese detail.

Occurrence

Manganes (Mn) is an essential element but it is toxic at higher concentrations. Pyrolusite, braunite, magnatite and tephroite are the minerals of Mn. Human beings get their daily quota of Mn from vegetables, fruits, nuts and germinal portions of grains. Mn is found in sea water at one ppb level.

Uses and pollution sources

Manganese is used in dry battery cells, electrical coils, ceramics, alloys and glass and steel manufacturing industries.

Functions of manganese

Some of the functions are as follows:

- Mn at low concentrations (2.5 to 5.0 mg) is essential for cell development.
- It acts as a cofactor in a number of enzymatic reactions, particularly those involved in phosphorylation, cholesterol and fatty acid synthesis.

Toxic effects of manganese

Some of its harmful effects are as follows:

- Higher level (100 ppm) of Mn accumulates in liver, kidney and bones. Manganese pneumonia is caused by excessive inhalation of Mn.
- Permanganate is the most toxic among different Mn forms. Mn^{2+} is three times more toxic than Mn^{3+}.
- Chronic exposure to Mn may cause manganese psychosis, an irreversible brain disease similar to Parkinson's disease. It is characterised by uncontrollable laughter, euphoria, sexual excitement, blindness, impulsiveness followed by impotence and speech disability.

VII. Mercury

Let us study mercury in detail.

Occurrence

Mercury, also named as quick silver, is extracted from cinnabar (HgS). Its natural abundance in soil is 0•1 ppm. Fossil fuels, coal and lignite contain 100 ppb of Hg. The ambient air concentration of mercury prevailing in some industrial areas is

NOTES

found to be 0•7 mg/m^3. The USEPA limits of Hg in drinking water is 2mgL^{-1}. Natural addition of Hg to the oceans is about 5000 tonnes per annum and a further 5000 tonnes is added via human activities. Sewage effluents may contain up to 10 times the level of Hg in natural water (0•001 ppm).

Uses and pollution sources

The largest consumer of Hg is chloralkali industry which manufactures Cl_2 and NaOH by using Hg electrodes. Production of electrical apparatus (bulbs, switches, batteries) and the use of Hg in agricultural industry (e.g., fungicides for seed dressings) constitute the second and third consumer of Hg. Pharmaceuticals, ointments, dental amalgams and finger print powders also disperse Hg in air.

Once mercury is absorbed on sediments of water bodies, it is slowly released into the water and constitutes a reservoir which is likely to cause chronic pollution long after the original source of Hg is removed.

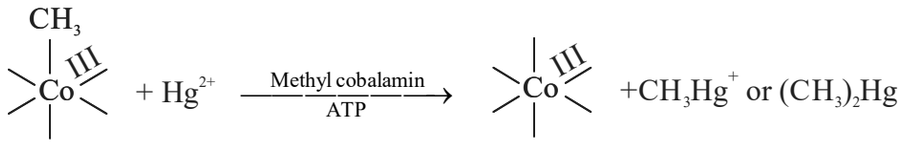
Toxic effects of mercury

Some of the harmful effects are as follows:

Minamata incident: The extreme toxicity of Hg was appreciated in 1953 when 52 persons living around Minamata Bay in Japan died of a Minamata disease. The victims had eaten shell fish contaminated with methyl mercury (27-102 ppm) containing effluent from a nearby vinyl chloride plastic company, Shin Nibon Chisso Hiryo. Several persons became crippled and showed genetic and neurological disorders. The causative agent, organomercurials was identified by Prof. Takeuchi.

Biological methylation of Hg in food chain

The Minamata chemical company discharged Hg into Bay but the fish were found to contain CH_3Hg^+. Actuaily Hg is readily scavenged and converted to CH_3Hg^+ by anaerobic methane synthesizing bacteria (clostridium). This conversion is facilitated by Co(III) containing viturnin B_{12} coenzyme. A CH_3^- group bonded to Co(III) on the enzyme is transferred enzymatically by methy1 cobalamin to Hg^{2+}, yielding CH_3Hg^+ or $(CH_3)_2Hg$.



An acidic medium promotes the conversion of $(CH_3)_2Hg$, to CH_3Hg^+ which is soluble in water. It is methyl mercury which enters the food chain through plankton and is concentrated by fish a factor of 10^4 as it passes through the food chain.

Toxicity of different chemical species of mercury

Some of the chemical species of mercury having harmful effects are as follows:

1. **Hg:** The vapours of elemental Hg are highly toxic when inhaled. Hg, being lipid soluble, can be absorbed through the intact skin. It attacks mainly liver, kidneys and can combine with carboxyl, sulphydryl, phosphoryl, amide and amino groups.

NOTES

2. **Hg^{2+}:** Mercuric ions are toxic but cannot easily transported across biological membranes. Hg^{2+} acts as potent enzyme inhibitors, protein precipitants and corrosive agents. Hg^{2+} ions can attach to sulphydryl groups present in haemoglobin and serum albumin. It can replace H atom from the sulphydryl group to form mercaptides of the type and $Hg(SR)_2$ where X is an electronegative radical and R is a protein. Higher levels of Hg^{2+} ions cause headache, diarrhoea, hemolysis, tremors and abdominal pain. Mercuric chloride is corrosive and when ingested precipitates proteins of the mucous membrane causing ashen appearance of the mouth, pharynx and gastric mucosa.
3. **Hg_2^{2+}:** Mercurous ions form insoluble Hg_2C1_2. Since a fairly high concentration of Cl is present in stomach so Hg_2^{2+} is not so toxic.
4. **Hg^+:** Methyl mercury is the most toxic species, It is soluble in fat, lipid and brain tissues, The covalent Hg-C bond is not easily disrupted and the alkyl mercury is retained in cells for prolonged time.

   The most dangerous aspect is the ability of RHg^+ to move through the placental barrier and enter foetal tissues.

   Attachment of Hg to cell membranes inhibits active transport of sugars across the membranes and allow the passage of K to the membrane. In case of brain cells, this will result in energy deficiency and disorders in the transmission of nerve impulses. It also causes irreversible damage to the central nervous system including cerebral palsy, mental retardation and convulsions. Methyl mercury poisoning at 0-5 ppm level leads to segregation of chromosomes, insomnia arid retardation in cell growth.
5. **R_2Hg:** Diorganomercurials have low toxicity but can be converted to RHW in acidic medium.

VIII. Arsenic

Let us study arsenic in detail.

Occurrence

Arsenic occurs in earth crust (2 ppm), sea water (5 ppb), soils (1 to 40 ppm), in human body tissues (18 mg) and blood (25 mg).

Uses and pollution sources

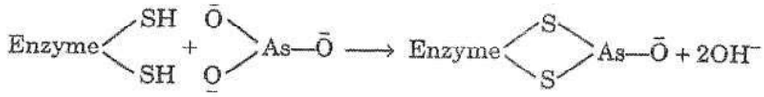
Arsenic oxide, known as white arsenic, is formed as a by-product in smelting of Ph, Cu and Ag ores. As_2O_3 is used in insecticides, as a preservatives and mordant in textile industry. Paris green (copper acetoarsenite) is used as feed additive. Arsenites are used as weed killers. Elemental arsenic is employed in the manufacture of infrared transmittance glass, semiconductors and oil cloth.

Toxic effects of arsenic

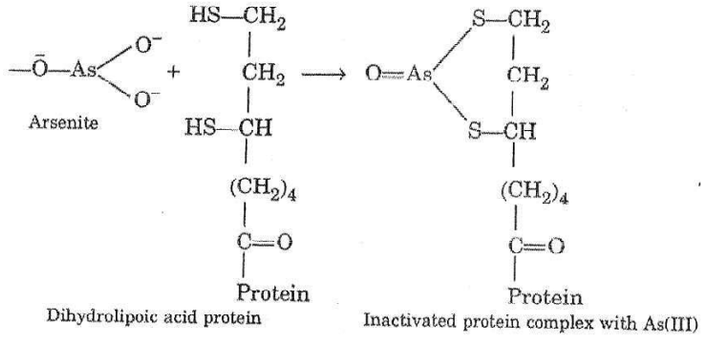
Arsenic is a general protoplasmic poison and it affects all the systems in man. The order of toxicity of arsenic compound is Arsines [As(III)) > arsenite [As(V)] > arsenate [As(V)] > arsenic organic acids [As(V)].

NOTES

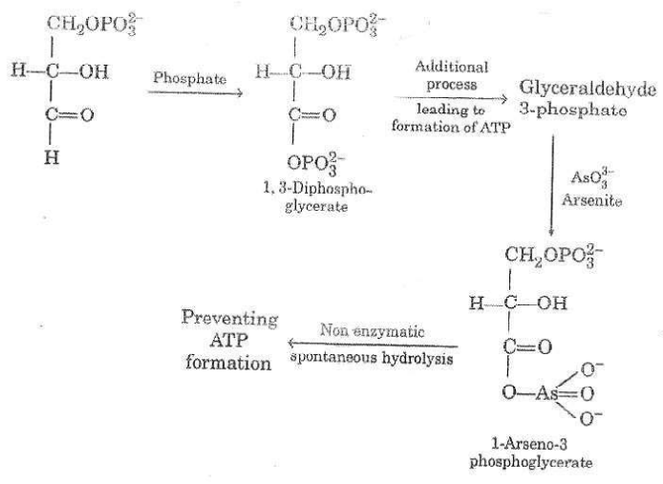
1. Complexation of As with coenzymes: As(III) exerts its toxic action by attacking SH group of an enzyme thereby inhibiting enzyme action.



The enzymes which generate cellular energy in the citric acid cycle are adversely affected. The inhibitory action is based on inactivation of pyruvate dehydrogenase by complexation with As(III) whereby generation of ATP is prevented.



By virtue of its chemical similarity to P, As interferes with some bio-chemical processes involving P. This is observed in the biochemical generation of the key energy yielding substance ATP (adenosine triphosphate). An important step in ATP generation is the enzymatic synthesis of 3-diphosphoglycerate from glyceraldehyde 3-phosphate. Arsenite interferes by producing 1-arseno-3-phosphoglycerate instead of 1, 3-diphosphoglycerate, Phosphorylation is replaced by arsenolysis which consists of spontaneous hydrolysis to 3-phosphoglycerate and arsenate.



2. Coagulation of proteins: As(III) compounds coagulate proteins possibly by attacking sulphur bonds maintaining the secondary and tertiary structures of proteins.

Soluble arsenicals are absorbed from mucous, membranes. Arsenic containing ointments or lipid soluble vesicants are absorbed through skin.

- Dermatitis and conjunctivitis are frequently observed in workers exposed to arsenic dusts. Continued inhalation of arsenic dust may cause perforation of the nasal septum.
- Slow arsenic poisoning causes nausea, salivation, vomiting, vascular failure, diarrhoea, hyperkeratosis, loss of weight, skin lesions and loss of hairs.
- Chronic ingestion of arsenic causes peripheral arteriosclerosis, known as black foot disease.
- Arsenic may cause peripheral neuritis resulting in motor and sensory paralysis of the nerve extremities. Arsenic is toxic to liver. It produces fatty infiltration and causes central necrosis, cirrhosis, hypoxic convulsions, vertigo and coma.
- It affects bone marrow, elements of blood and can cross placenta, membranes.
- The fatal cases of arsenic poisoning are mostly homicidal, a few are suicidal and accidental.

4.6 GENERAL SURVEY OF INSTRUMENTAL TECHNIQUES FOR ANALYSIS OF HEAVY METALS IN AQUEOUS SYSTEMS

The various methods employed for the monitoring of metal pollutants from the atmosphere and aqueous sources include atomic absorption spectrophotometer (AAS), atomic emission spectroscopy (SAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), ion-selective electrodes, fluorescence, plasma spectroscopy, neutron activation analysis (NAA), thermogravimetric analysis (TGA), etc. Some of these instrumental techniques along with their sensitivities are listed in Table 4.1:

Table 4.1 *Some Sensitive Instrumental Techniques*

Method	Sensitivity (mole L^{-1})
Anodic stripping voltammetry (ASV)	$10^{-9} – 10^{-10}$
Atomic absorption spectrophotometry (AAS)	$10^{-6} – 10^{-7}$
Optical and emission spectroscopy	$10^{-5} – 10^{-6}$
Classical polarography	$10^{-5} – 10^{-6}$
Potentiometry with ion-selective electrodes	$10^{-4} – 10^{-5}$

The comparative detection limits for some heavy metals is given in Table 4.2.

NOTES

Table 4.2 Comparative Detection Limits (ppb) C (μg)L)]

Metal	Flame AAS	Graphite AAS	ICPAES
As	100	0.06	15
Co	5	0.03	2
Cr	3	0.005	2
Mn	3	0.004	0.5
Pb	10	0.03	15
Zn	0.6	0.007	1
Cd	1	0.008	1
Cu	2	0.008	2
Fe	5	0.003	1
Ni	8	0.02	5
Ti	50	0.30	1
Se	100	0.10	15

Some of these instrumental techniques are discussed in brief in the following sections.

4.6.1 Atomic Absorption Spectrophotometry for Heavy Metal Analysis

Let us study the method in detail.

Princple

Analysis of Cu, Fe, Zn, Mn metals using AAS was given by Lindsay and Norvell. Diethylene triamine pentacetic acid (DTDA), a chelating ligand, combines with free metal ions (Cu^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}) in the aqueous system to form soluble complexes. Stability constant for the simultaneous complexes of Cu, Fe, Zn, Mn shows that DTPA is a most suitable extractant. The concentration of these metal ions are determined by using AAS.

Apparatus

The apparatus used in the method are as follows:

- Atomic absorption spectrophotometer
- Hollow cathode lamps of Cu, Fe, Zn, Mn
- Volumetric flasks and pipettes etc.

Reagents

The reagents are as follows:

1. **DTPA solution (0.005 M):** Take 1.967 g of DTPA in 1 L volumetric, flask and make up the volume to the mark with deionised water.
2. **Stock standard solution:** Dissolve 0.1 g of the metal foil in dilute HC1 (1 : 1) and make the volume to one litre with deionised water to obtain 100 μg/L (i.e., mg/L or ppm) of solution of every metal ion. Alternatively, dissolve separate the following:

   0.3928 g of $CuSO_4.5H_2O$ 0.4964 g of $FeSO_4.7H_2O$

   0.3076 g of $MnSO_4.H_2O$ 0,4398 g of $ZnSO_4.7H_2O$

in 1 L of deionised water to prepare 100 mg/ mL of stock solutions of Cu, Fe, Mn and Zn, respectively. Add 5 mL of H_2SO_4 to the solutions.

3. **Working standard solutions:** Transfer 0, 1, 2, 4, 6 and 8 mL of stock solution (100 mg M^{2+}/mL or 100 ppm of M^{2+}, where $M^{2+} = Cu^{2+}$, Fe^{2+}, Mn^{2+}, Zn^{2+}) to a series of 100 mL volumetric flasks. Add DTPA. solution and make up the volume to the mark. This will give standard solutions having metal ion concentration of 0, 1, 2, 4, 6 and 8 mg/mL, (or ppm).

NOTES

Procedure

The method involves the following steps:

1. For the determination of Cu^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+} in aqueous sample, set the atomic absorption spectrophotometer to zero using blank solution.
2. Feed standards of the metals to be determined to AAS to standardise the instrument to read absorbance or concentration in the sample having the given metal within the standardised range.
3. Feed the DTPA extract and record the absorbance concentration of the metal.
4. Repeat the above steps for every metal.
5. In case the AAS shows a sign of error for some metal in a particular sample indicating thereby that the sample has a concentration out of the range for which the instrument has been standardised then make further dilution of the sample 2-5 times. Feed the sample and record absorbance or concentration.

Calculations

Most of the modern AAS are calibrated to display the concentration of a metal in ppm directly in the sample. In such cases, the concentration of a given metal in the sample is calculated by multiplying the displayed reading by the dilution factor 2. If the AAS displays the reading in absorbance, then a standard curve has to be prepared for the known standards on a graph paper. Convert the absorbance readings into concentration (mg/mL) from the standard curve. The amount of given metal is then calculated as follows:

Volume/weight of the sample taken = 10 mL
Volume of DTPA extractant added = 20 mL
Dilution = 2 times
Absorbance shown by AAS = A

Concentration of the metal as read from the standard curve against A = C (or mg/mL (or mg/kg = ppm).

Precautions

Some of the precautions need to be taken are as follows:

- Apparatus (glass/polythene) to be used for analysis must be thoroughly washed with acidified water and deionised water water.

- Turbid solutions should not be used for feeding since they may block the capillary of AAS.

4.6.2 Analysis of Copper by Different Techniques

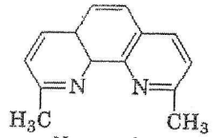
NOTES

The different techniques are as follows:

1. Spectrophotometric method

This method involves the following:

- **Cuprethol method:** Cupric ions yield a yellow chelate when react with cuprethol (bis-2-hydroxy ethyl) dithiocarbamates at a pH of 5 which is maintained by HCl and CH_3COONa solution, The interference from Fe is removed by adding pyrophosphate. The yellow colour is measured spectrophotometrically.
- **Neocuproine method.** Copper reacts with neocuproine (2, 9-dimethyl 1, 10-phenanthroline) in weakly acidic solution (pH 3 to 8) to form a complex which can be extracted as a yellow coloured solution N into a mixture of $CHCl_3$ and CH_3OH. The yellow coloured solution is measured spectrophotometrically at 457 nm.



Neocuproine

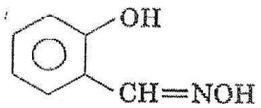
Procedure

To 100 mL sample in a 250 mL beaker add 1 mL conc. H_2SO_4 and 5 mL conc. HNO_3. Evaporate to white dense fumes of SO_3. Add 5 mL conc. HNO_3 and heat to fumes until the solution becomes colourless. Cool the solution, add 70-80 mL of distilled water and again boil. Cool and filter the solution in a 100 mL volumetric flask and make the volume to 100 mL by distilled water.

Transfer 50 mL of this solution into a 150 mL separatory funnel and dilute it with 50 mL of distilled water. Add 5 mL, of 1% hydroxyl amine hydrochloride, 10 mL of 40% sodium citrate and 10 mL of neocuproine, reagent (100 mg/100 mL alcohol, CH_3OH), maintaining the pH between 4 to 6. Extract the solution with $CHCl_3$ and repeat the extraction of aqueous layer with 10 mL chloroform. Transfer the $CHCl_3$ extract in a volumetric flask containing $CHCl_3$ extract. Dilute the combined extract to 25 mL with methanol. Measure the absorbance of the coloured solution at 457 nm against a reagent blank.

2. By salicylaldoxime

Dissolve 1 g salicylaldoxime in 5 mL of alcohol and dilute to 100 mL with water. The neutral solution of the sample reacts with a drop of 10% acetic acid and a drop of salicylaldoxime, yielding green precipitate. The sensitivity is 0.0005 mg of Cu in 10 ppm concentration.



Salicylaldoxime

NOTES

3.Atomic absorption method

Aspirate Cu^{2+} solution into an air-acetylene flame of an atomic absorption spectrophotometer and measure the absorbance at 325 mm.

4.6.3 Analysis of Cu and Zn by Differential Pulse Polarography (DPP)

Most tap waters contain sufficient Cu and Zn for them to be determiend directly from DPP. Since the sample contains very less interferring species, the experiment may be conducted without any sample preparation. The experiment may be conducted by comparing the response of the sample against a calibration curve prepared from standard solutions of the two metals or it may use the method of standard additions made directly to the sample.

Typical conditions

Drop time 1 sec, starting potential + 0.1 V (Vs SCE), scan range 1.4 V negative, scan rate 2 mVs^{-1}, pulse modulation amplitude 50 mV, current range micro. Ampere full scale deflection.

Method

Prepare solutions which contain both Zn^{2+} and Cu^{2+} in the range 0.5 to 20 mg^{-1} in a supporting electrolyte of 0.01 M KCl. Other supporting electrolyte such as an ethanoate or citrate buffer at 0.05 to 0.2 M can be used but the material must be low in heavy metals. Place a sample of the lowest concentration in the polarographic cell and degas for the recommended time, usually 2 minutes. Wait for one minute so that the solution becomes stationary and record the DPP trace. The copper peak may well be on the side of a large peak at positive potentials but should be fairly well resolved, whereas the zinc peak should be completely symmetrical. If the peak heights are satisfactory, record traces for each of the other standard solution in the same way.

Construct as calibration curve of peak height against concentration for the two metals. (If the peaks are too large or small, adjust the sensitivity of the apparatus so that all the traces can be conveniently observed. Obtain traces for the tap water sample under the same conditions and with the addition of sufficient 0.1 M KC1 solution to the sample to give the same final concentration of supporting electrolyte as the standard solutions. Measure the peak heights for both copper and zinc in this solution. By comparison against the standard curve, estimate the concentration of copper and zinc in tap water.

NOTES

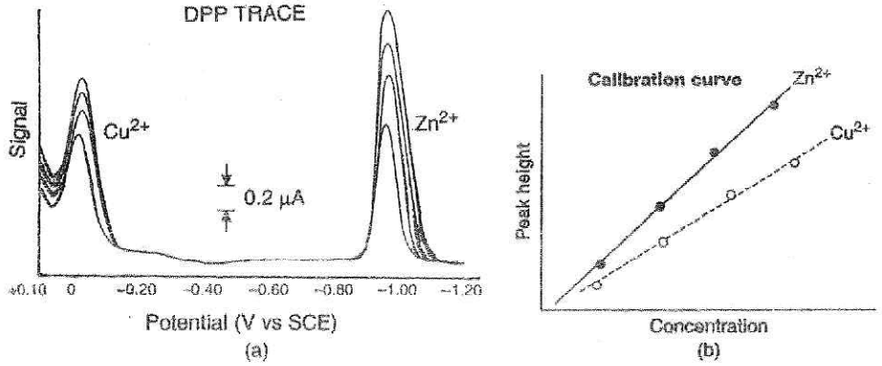


Fig. 4.1 *(a) Copper and zinc in tap water.*
(b) Calibration graph of peak height against concentration

Square wave polarography for the determination of copper and zinc in tap water

Typical experimental conditions

Drop time is for single hanging drop. Scan increment 2 mV. Square wave frequency 100 Hz. Starting potential +0.1 V (Vs SCE). Scan rate 200 mVs^{-1}. Scan range 1.4 V negative. Pulse modulation amplitude 50 mV. current range 2μA full scale deflection.

This experiment will take 7s per scan compared with 700s or nearly 12 minutes for the standard DPP scan.

4.6.4 Analysis of Lead in Water

The analysis of lead in water can be done by using the following methods:

1. **Atomic absorption method:** Lead can be measured by directly aspirating the sample into an air acetylene flame and measuring the absorbance of Pb at 283.3 nm. For lower concentration of lead follow, the procedure of complex formation with ammonium pyrrolidine dithiocarbamate, extraction into methyl isobutyl ketone and then aspiration of the methyl isobutyl ketone complex into the flame (air-acetylene) of an atomic absorption spectrophotometer.
2. **Polarographic method:** The half wave potential ($E_{1/2}$) values are – 0.37 to - 0.40 V, - 0.43 to - 0,47 V and - 1.13 to - 1.17 volts in 0.18 M $(NH_4)2SO_4$, 0.4 M NH_4OH, + 0.18 M $(NH_4)_2SO_4$ and 0.44 M NH_4OH + 0.18 M $(NH_4)_2SO_4$) + EDTA respectively.

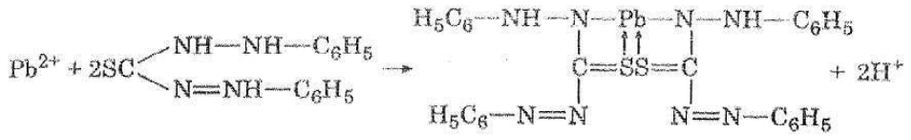
Procedure

Take 20-50 mL sample in a flask and add 10 mL of $KMnO_4$ solution. Add 3 mL of conc. H_2SO_4 and reflux the mixture for at least 3 hours. Now cool and add 40% hydroxylamine hydrochloride ($NH_2OH.HCl$) solution in order to reduce $KMnO_4$. Filter and dilute the filtrate to about 100 mL by adding distilled water.

Now take 20 mL aliquot portion of the solution in a separatory funnel and add 10 mL, of 40% sodium citrate solution and then add NH_4OH solution to adjust the pH to 8.5. Extract it with 0.01% dithizone in CCl_4, solution. Back

extract with 1% HNO_3 and transfer to a 25 mL volumetric flask. Add 2.0 mL of 1 M KCl solution and HNO_3 to adjust pH to 6. Make up the volume to the mark, Measure lead polarographically at $E_{1/2} = 0.4$ volts.

3. **Spectrophotometric dithizone method:** Lead ions react with dithizone in chloroform to form a complex which is soluble in chloroform and imparts cherry red colour. The red extract is measured at an absorbance of 515 nm against a reagent blank.



4.6.5 Other Techniques for Heavy Metals Determination

Some of the other techniques used for the determination of heavy metals in various samples are discussed below in brief.

1. **Atomic emission spectroscopy (AES) or flame photometery:** The use of a gas flame as a source of excitation for atomic emission is known as flame photometry or atomic emission spectroscopy (AES). This technique is mainly used for the determination of alkali and alkaline earth metals. This technique uses the intensity of light emitted from a flame, plasma, arc, or spark at a particular wavelength to determine the quantity of an element in a sample. The wavelength of the atomic spectral line in the emission spectrum given the intensity of the element while the intensity of the emitted light is proportional to the number of atoms of the element.
2. **Inductively coupled plasma – atomic emission spectroscopy (ICP-AES) technique:** This method uses an inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wevelengths characteristic of a particular element. Advantages of this technique are the excellent limit of detection and linear dynamic range, multielement capability, low chemical interference and a stable resproducible signal.
3. **Hydride Generations (HG) Technique:** This a very effective analytical technique developed to separate hydride forming metals, such as Se and As, from a range of matrices and varying acid concentrations. This method enhances for these elements by a factor of 10 to 100.
4. **Cold Vapour Atomisation Technique:** This method is applicable for the determination of mercury because it is the only metal that has an appreciable vapour pressure at ambient temperature.

NOTES

Check Your Progress

8. Define heavy metals.
9. Which mineral is essential for the normal biological activities of amine oxidase and tyrosinase enzymes?
10. Name the diseases caused by cadmium toxicity.
11. Where does chromium occur in the earth crust?
12. List the uses of manganese.

4.7 MEASUREMENT OF DISSOLVED OXYGEN (DO), CHEMICAL OXYGEN DEMAND (COD) AND BIOCHEMICAL OXYGEN DEMAND (BOD)

Let us study dissolved oxygen (DO), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in detail.

4.7.1 Dissolved Oxygen (DO)

Dissolved oxygen refers to the level of free non-compound oxygen present in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a water body. The methods most commonly used for DO measurement is Winkler method, polarographic method and membrane electrode method. These methods are discussed below.

1. Modified winkler method

Let us study the method in detail.

Principle

KI is oxidised to l_2 with the dissolved oxygen present in the water sample after adding $MnSO_4$, NaOH and Kl. The basic manganeic oxide formed from NaOH and $MnSO_4$ acts as an oxygen carrier to enable the dissolved oxygen in the molecular form to take part in the reaction. The DO present in the sample oxidises the Mn^{2+} to its higher valency which precipitates as a brown hydrated oxide after the addition of NaOH and KI. On acidification, the manganese reverts back to the divalent state and an equivalent amount of iodine is liberated from the KI present. This liberated iodine is titrated against standard sodium thiosulphate (hypo) solution, using starch as indicator.

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$

$$2Mn(OH)_2 + H_2SO_4 \longrightarrow \underset{\text{Basic manganeic oxide}}{2MnO(OH)_2}$$

$$MnO(OH)_2 + H_2SO_4 \longrightarrow MnSO_4 + 2H_2O + O$$

$$2Kl + H_2SO_4 \longrightarrow K_2SO_4 + H_2O + I_2$$

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

Interferences

Iron, nitrite and microbial mass are the chief sources of interference in this method. The interference due to nitrite can be eliminated by adding sodium azide.

NOTES

$$\underset{\text{Sodium azide}}{2NaN_3} + H_2SO_4 \longrightarrow \underset{\text{Hydrazoic acid}}{2HN_3} + Na_2SO_4$$

$$HNO_2 + NH_3 \longrightarrow N_2O + N_2 + H_2O$$

Reagents required

The required are as follows:

- **Standard N/20 $K_2Cr_2O_7$ (Mol. wt. 294.21; Eq. wt. 49.035):** Dissolve 2.4518 g of dry A.R. $K_2Cr_2O_7$ powder in distilled water and make up to 1000 mL in a measuring flask. For more accurate work, the $K_2Cr_2O_7$ powder should be dried at 140°C for 1 hour in an electric oven and cooled in a desiccator before weighing.
- **N/20 Sodium thiosulphate solution:** Dissolve 12.41 g of A.R. sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) in 1 L of distilled water. The solution may be preserved by adding 3 drops of chloroform.
- **Conc H_2SO_4.**
- **Manganese sulphate.** Dissolve 400 g of $MnSO_4$ or 480 g of $MnSO_4$, $4H_2O$ in distilled water and make up to 1 L. This solution should not give colour with acidified potassium iodide and starch.
- **Alkaline iodide-azide reagent.** Dissolve 500 g of NaOH and 150 g of KI in distilled water. Add 10 g of NaN_3 in 40 mL distilled water. Dilute to 1 L. This solution should not give colour with starch on dilution and acidification.
- **Starch solution:** Prepare a fresh paste of 0.5 g of starch with distilled water and pour this into 100 ml of boiling distilled water while stirring.
- Solid KI free from iodate, $NaHCO_3$ and conc HCl.

Procedure

The method involves the following steps:

(A) Standardization of hypo solution: Transfer 100 ml, of boiled, cooled distilled water in a 250 mL iodine flask. Add about 2 g of KI, 2 g of $NaHCO_3$ and shake until the salts are dissolved, Add 6 mL of conc. HCl slowly while gently rotating the flask. Add 25 mL of N/20 $K_2Cr_2O_7$ solution and mix the solutions well. Wash the inner sides of the flask with a little distilled water and stopper the flask. Allow to stand for 5 minutes for the completion of the reaction.

$$Cr_2Or\,O_7^{2-} + 6I^- + 14H+ \longrightarrow 2Cr3+ + 3I_2 + 7H_2O$$

Rinse the stopper with distilled water and titrate the liberated iodine with the hypo solution from the burette. When most of the iodine has reacted, the solution acquires a greenish-yellow colour. At this stage, add 1 mL of starch solution when the solution turns blue due to the formation of starch iodine complex. Continue the

NOTES

titration until the greenish blue colour changes to light green by the addition of a single drop of hypo at the end point.

Note. If KI is not iodine free; a blank experiment has to be performed and the corresponding correction should be made to the titre value.

(B) Titration with the sample: For the DO determination, the sample should be collected from below the surface at a known depth and without aeration. Generally, the sample is collected in a BOD bottle with a DO sampler.

For laboratory practice, collect the water sample in a 300 mL BOD bottle, avoiding contact with air. Add with the help of a pipette 2 mL of $MnSO_4$ followed by 2 mL of the alkaline iodide-aside reagent in such a way that the tip of the pipette should dip below the liquid surface while adding the reagents. Stopper the bottle immediately and mix well by inverting the bottle 3 to 4 times, Allow to stand for 2 minutes, add 1 mL of conc. H_2SO_4, insert the stopper and mix well till the precipitate goes into solution, Allow to stand for 5 minutes. Take 203 mL of the clear solution into a conical flask and titrate against hypo solution using starch as indicator.

Note. When 2 mL of $MnSO_4$ + 2 ml of alkali iodide-aside reagent are added into full BOD bottle, from below the liquid surface, 4 mL of the original water sample is lost. Hence 203 mL now taken for titration will correspond to 200 mL of the original sample.

$$\frac{200 \times 300}{(300-4)} = 203 \text{ mL}$$

Calculation

20 mL of N/20 $K_2Cr_2O_7 \equiv$ 20 mL of N/20 hypo

$$\therefore \quad \text{Normality of hypo} = 20 \times \frac{1}{20} \times \frac{1}{20} \text{ N} = \frac{\text{N}}{20}$$

DO in 200 mL of the water sample, on treatment, liberates iodine, which on titration is found to be equivalent to 4.2 mL of N/20 hypo.

$\therefore$ Normality of the water sample with respect to DO

$$= 4.2 \times \frac{1}{20} \times \frac{1}{200}$$

$$\therefore \quad \text{Strength} = 4.2 \times \frac{1}{20} \times \frac{1}{200} \times 8 \text{ g/L} = 0.0084 \text{ g/L}$$

$$= 8.4 \text{ mg/L} = 8.4 \text{ ppm.}$$

Result

Dissolved oxygen in the given water sample = 8.4 mg/L or 8.4 ppm.

Alternative method of calculations

For 200 mL of the original water sample used, calculate on the basis that]

1 mL of 0.05 N hypo $\equiv$ 2 mg of DO/L

$\therefore$ 4.2 mL of 0.05 N hypo $\equiv 2 \times 4.2$

= 8.4 mg DO/L in water sample

2. Polarographic method

O_2 can be reduced at various electrodes in aqueous solutions if a small negative voltage is applied. The magnitude of the current which flows is determined by the rate at which O_2 can diffuse to the electrode.

3. Membrane of electrode method (Mackereth Oxygen cell for the determination DO)

The polarographic method is not desirable for DO analysis in domestic/industrial waste waters as the Hg electrode gets poisoned by impurities in the test solutions. This problem is solved by using the membrane electrode method. Two metal electrodes, one of Ag and the other of Pb are immersed in a saturated $KHCO_3$ solution separated from the test solution by a polyethylene membrane. Thus, a galvanic cell can be plugged to a pH meter to give a direct reading of DO in mg L^{-1} (the scale of 0 to 14 pH becomes 0 to 14 mg L^{-1} DO). the current is measured for sample, for a standard (sample after air saturation) and for a blank (sample after treatment with a little Na_2SO_3 to expel O_2).

$$O_2 + 2H_2O + 4e^- \longrightarrow 4OH^- \text{ (Ag electrode)}$$

$$Pb + 4OH- \longrightarrow PbO_2 + 2H_2O + 4e^-$$

or

$$2Pb + 4OH- \longrightarrow 2Pb(OH)_2 + 4e^- \text{ (Pb electrode)}$$

4.7.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is the amount of oxygen needed to oxidize the organic matter present in water. COD test is also used to determine the amount of inorganic chemicals in a sample. COD test is generally performed on wastewater. The pollution level is calculated by meaning the amount of organic matter in the water. Water with excess of organic material can have a negative effect on the environment in which the wastewater is discharged. CO can be determined as explained in the below mentioned method.

Principle

The chemical oxygen demand (COD) is a measure of the oxygen equivalent of that portion of organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. This is an important and quickly measured parameter for stream and industrial waste water studies and control of waste treatment plants.

Most types of organic matter are completely oxidize by a boiling mixture of chromic and sulphuric acid to produce CO_2 and H_2O. A measured quantity of the sample is refluxed with a known amount of $K_2Cr_2O_7$ and H_2SO_4.

$$3\{CH_2O\} + 16H+ 2Cr_2O_7^{2-} \longrightarrow 4Cr^{3+} + 3CO_2 + 11H_2O.$$

The excess of dischromate remaining unreacted is titrated with ferrous ammonium sulphate solution. The $K_2Cr_2O_7$ consumed is proportional to the amount of oxidizable organic matter measured as oxygen equivalent.

NOTES

Reagents

The reagents required in this method are as follows:

1. **Standard 0.25 N $K_2Cr_2O_7$ solution:** Dissolve 12.259 g of pure and dry AR $K_2Cr_2O_7$ in distilled water and dilute to 1 litre. Add about 120 mg of sulphamic acid to eliminate any interference because of nitrites that may be present in the waste water sample upto 6 mg/L.
2. **Sulphuric acid-silver sulphate reagent:** Add 5.5 g of Ag_2SO_4 to 1 kg or conc. H_2SO_4 and keep over night for dissolution.
3. **Standard 0.1 N $Fe(NH_4)_2 (SO_4)_2 . 6H_2O$ solution:** Dissolve 39 g of the pure salt in distilled water. Add 20 mL of conc. H_2SO_4 and dilute to 1 litre.
4. **Ferroin indicator:** Disolve 1.485 g of 1, 10-phenanthroline monobydrate with 0.695 g of pure $FeSO_4$ $7H_2O$ in water and dilute to 100 mL.
5. **$HgSO_4$ AR grade.**

Procedure

Place 0.4 g of $HgSO_4$ in reflux flask and add 20 mL of the sample or an aliquot of the sample diluted to 20 mL with distilled water. Mix well and add 10 mL of 0.25 N $K_2Cr_2O_7$ solution. Drop some pumice stones and slowly add 30 mL of H_2SO_4-Ag_2SO_4 reagent while continuously swirling the flask. If the colour changes to green add more $K_2Cr_2O_7$ and H_2SO_4—Ag_2SO_4 reagent or alternatively discard the solution and take fresh sample with lesser aliquot. Mix the contents of the flask thoroughly. Connect the flask to the condenser and slowly heat. Reflux for at least 2 hours. Cool and wash down the condenser with distilled water such that the washings fall into the flask. Dilute to about 150 mL, cool and titrate the unreacted $K_2Cr_2O_7$ with N/10 ferrous ammonium sulphate solution using ferroin as indicator. The colour change at the end point is from blue green to wine red. Perform a blank experiment with distilled water instead of the water sample.

Calculations

The COD of the sample is calculated as follows:

$$\text{COD in mg/L} = \frac{(V_1 - V_2)\, N \times 800}{x}$$

where V_1 = Volume of ferrous ammonium sulphate run down in the blank experiment

V_2 = Volume of ferrous ammonium sulphate run down in the test experiment

N = Normality of ferrous ammonium sulphate solution and

x = Volume of test sample taken

4.7.3 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is the amount of dissolved oxygen (DO) needed by aerobic biological organism to break down organic material present in a given water time period. BOD symbolizes the amount of organic pollution present

in an aquatic ecosystem. Higher the levels of organic matter in water bodies, the greater the BOD. The method for determinging BOD is discussed below.

Principle. The dissolved oxygen content of the sample is determined before and after five days incubation at 20^0C. The BOD is calculated on the basis of the amount of oxygen depleted.

NOTES

Reagents

The reagents are as follows:

- **Calcium chloride solution:** Dissolve 27.5 g of anhydrous $CaC1_2$ or equivalent amount of the hydrated salt in distilled water and dilute to 1 L.
- **Magnesium sulphate solution:** Dissolve 27.5 g of $MgSO_4.7H_2O$ in distilled water and dilute to 1 L.
- **Ferric chloride solution:** Dissolve 0.25g $FeC1_3.6H_2O$ in distilled water and dilute to 1 L.

(iv) **Phosphate buffer solution:** Dissolve 8.5 g of KH_2PO_4, 21.75 g of K_2HPO_4, 33.4 g of $Na_2HPO_4.7H_2O$ and 1.7 g of NH_4Cl in 500 mL distilled water and make upto 1 L. The pH of this solution should be 7.2. Keep this solution in refrigerator to prevent any biological growth.

(v) $MgSO_4$. solution, alkali-iodide-azide reagent, H_2SO_4, etc. are prepared as in DO determination.

Preparation of dilution water

Redistil water in presence of a little alkaline permanganate. Store this double distilled water in a BOD incubator at 20^0C. Aerate the required quantity of this distilled water at 20°C with clean compressed air. Add 1 mL each of $CaCl_2$, $MgSO_4$, $FeCl_3$ and phosphate buffer solutions per litre to the above aerated distilled water and mix thoroughly. This standard dilution water should be prepared just before use.

The addition of small measured volume of water containing a good bacterial population to the dilution water is called seeding. Seeding is not required for sewage and sewage effluents because they contain the bacterial flora. Seeding is necessary for industrial effluents which generally do not contain any bacteria. The seeding material that is generally used is fresh and settled raw sewage or fresh and settled final effluent of an aerobic biological process. The seed should be kept for 1 to 2 days at 20°C before use. The seed concentration recommended is 1 to 2 mL per litre of dilution water.

Pre-treatment methods

The methods involve the following conditions:

1. If the pH of the sample is not in the range of 6.5 to 8.5, add the necessary quantity of $1NH_2SO_4$ or 1NNaOH to bring the pH in the said range. Determine the amount of H_2SO_4 or NaOH so added on a separate aliquot of the sample.
2. If the sample contains any chlorine, destroy the chlorine by adding a known excess of freshly prepared 0.025 N sodium sulphite solution. Determine

NOTES

the amount of the Na_2SO_3 consumed by the iodometric method on a separate aliquot of the sample.

3. Samples must be thoroughly shaken just before dilutions are made.
4. Samples stored in refrigerator should be allowed to reach room temperature before making the dilutions.
5. If the BOD of a polluted river water containing algae is to be determined, the algae must be separated by centrifugation and the centrifugate should be used for the BOD test.
6. Some waters, e.g., those containing algae may be supersaturated with dissolved oxygen. In such cases, the excess oxygen above the saturation level should be removed by partly filling the sample in a bottle and agitating or aerating at 20^0C.

Procedure

It is preferable to make a series of dilutions for a sample such that at least three of the dilutions should deplete 20% to 90% of the initial dissolved oxygen (DO). The COD value or permanganate value may be treated as guideline for the purpose of dilution. The following guidelines are advantageous :

Table 4.4 *Guidlines*

S.No.	Sample	% of the sample in the dilution mixture	Vol. of sample per litre of the dilution mixture, mL.
1.	Raw sewage	5 to 0.5	50 to 5
2.	River waters	100 (i.e., no dilution)	1000
3.	Polluted river waters	50 to 10	500 to 100
4.	Industrial effluents	20 to 0.05	200 to 0.5

The following dilution technique may be used as follows:

- Transfer carefully the standard dilution water (or the seeded dilution water in case of trade wastes) into a 1 L graduate glass cyclinder until it is half full without any air entrainment. Now add the appropriate quantity of the well mixed sample, into the glass cylinder without producing air bubbles. Make up the volume to the mark using the dilution water. Mix well with a glass rod without air entrainment. Siphon the mixture into two BOD bottles of 300 mL capacity. Fill the two BOD bottles carefully without allowing any air bubble and seal the bottles.

  Prepare the other dilutions of the sample by adopting the above procedure. Also, siphon out the standard dilution water (or the seeded dilution water) into two BOD bottles and water seal them after filling them completely.
- Utilize one set of the entire series of dilutions prepared above for the immediate determination of dissolved oxygen (DO). Keep the other set in a BOD incubator maintained at 20T for 5 days. After 5 days determine the DO content of all the incubated samples.

Calculations. Calculate the BOD values using the following relation.

$$(\text{BPD mg/L (5 days at 20°C)} = \frac{(DO_0 - DO_5 - B) \times 100}{\% \text{ of the sample used}}$$

where DO_0 = Initial DO content in mg/L

DO_5 = DO content after incubation for 5 days.

B = Blank correction determined by the difference between the DO contents of the blank on the initial day and that after 5 days incubation.

Precautions

Some of the precautions are as follows:

- The temperature of 20°C should be constantly maintain during the 5 days incubation period. Even an increase or decrease of temperature by 1°C may increase or decrease the BOD value by about 4.7%.
- The BOD test should be performed as soon as possible after the sample collection.

NOTES

4.8 PESTICIDES AS WATER POLLUTANTS

The pesticide is a composite term that includes all chemical that are used to kill or control pests. In agriculture, this includes herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (vertebrate poisons).

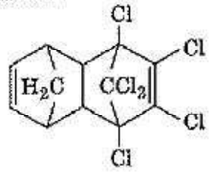
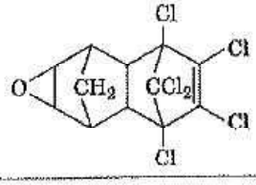
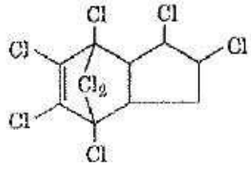
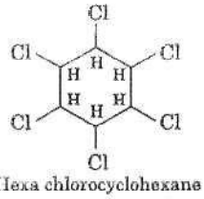
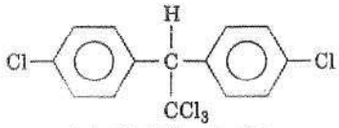
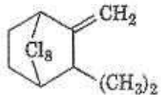
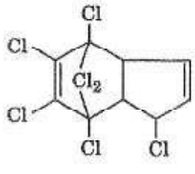
A fundamental contributor to the green revolution worldwide has been the development and application of pesticides for the control of a wide variety of insectivorous and herbaceous pests that would otherwise dimish the quantity and quality of food produce. Unfortunately, with the benefits, pesticide have some harmful effects, some are so serious that they now threaten the long term survival of the major ecosystems by disruption of predator-prey evaluation ships and loss of biodiversity. Also, pesticides can have serous health consequences for humans too. They may lead to cancer, pulmonary and haematological morphidity, as well as an inborn deformities and immune deficiencies.

4.8.1 Classification of Pesticides

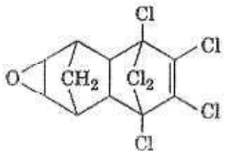
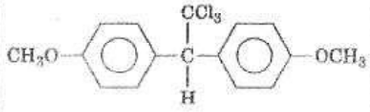
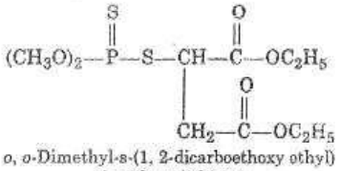
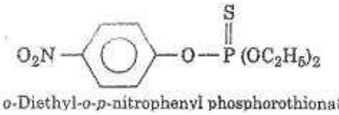
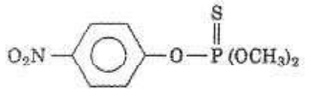
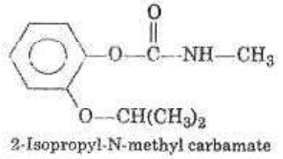
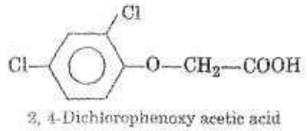
Among the pesticides, herbicides are used to kill weeds, fungicides are toxic to fungi, insecticides kill insects, rodenticides are used against cats and mice, nematicides in hibit nematodes. Molluscides are used to kill mollus (snails and slugs), piscicides are used to control undesirable fish species. Structural classification, used and the permissible limit of various pesticides are summarized in Table 4.3.

NOTES

Table 4.3 *Structure and Uses of Pesticides*

Trade name	Formula	Uses	Permissible limit
I. *Chlorinated hydrocarbon*			
Aldrin		Soil insecticide for control of nuts, beetles and cotton pests. Use is now banned in USA.	0·003 µg/L
Dieldrin			
Chlordane		Effective against termites. Potential carcinogen. Use banned in USA in 1975.	0·01 µg/L
Lindane	 Hexa chlorocyclohexane	Control of cotton insects and rice stem borer.	0·01 µg/L
DDT	 1, 1, 1-Trichloro-2, 2-bis (*p*-chlorophenyl)-ethane	Broad spectrum-cotton soya- bean and peanut pests, mosquito control. Persistent in the environment. Accumulates in food chain, use banned in USA.	0·001 µg/L
Toxaphene		Insect control on crops and livestock; widely used in USA, ban has been proposed for carcinogenic properties.	5 µg/L
Heptachlor		Pest control in soil; use suspended due to potential carcinogenicity.	0·001 µg/L

NOTES

Endrin		Effective against black current mud mite, also used as Zoocide. Precautions to be taken to avoid skin contact during application.	0·004 µg/L
Metho-xychlor		Popular DDT substitute, reasonably biodegradable, low-toxicity to mammals.	0·03 µg/L
II. *Organophosphates*			
Malathion	 o, o-Dimethyl-s-(1, 2-dicarboethoxy othyl) phosphorodithioate	Control some pests of fruits and vegetable-little hazard to mammals.	0·1 µg/L
Parathion	 o, o-Diethyl-o-p-nitrophenyl phosphorothionate	Larvicide for mosquito control, also broad spectrum insecticide for fruit and vegetable pests.	0·001 µg/L
Methyl parathion		Control of plant pests; ranks second in pesticide consumption in USA.	0·001 µg/L
III. *Carbamates*			
Carbaryl (Sevin)	 1-Naphthyl-N-methyl carbamate	Used on crops-cotton, forage, fruits and vegetables; lawn and garden insecticide; low toxicity to mammals.	0·005 µg/L
Baygon	 2-Isopropyl-N-methyl carbamate	Control of flies, mosquitoes, ants and cockroaches.	0·001 µg/L
IV. *Chlorophenoxy acids*			
2, 4-D	 2, 4-Dichlorophenoxy acetic acid	Herbicide-control of broad leave weeds, aquatic, vegetation; military defoliant (May contain highly toxic TCDD as impurity)	100 µg/L

NOTES

4.8.2 Sources of Pesticidal Pollutants in Water

Some of the common sources are as follows:

- **Rain water:** Pesticides contaminate in vapour phase in air, gets adsorbed onto the dust particles which ultimately reach soil or water along with the rain water. Weibel et al., recorded 187 mg/L of total DDT of which 18 mg/L was DDE in rain water of Ohio, USA. Residues of BHC, DDE and dieldrin are detected in rain water.
- **Spray drift:** Water contaminates heavily (40%) with pesticides during aerial spray. Wind also carry the drift to considerable distances.
- **Run off from agricultural fields:** Caro and Taylor have reported that 0.07% of dieldrin applied to the soil persists in run off water. DDT in water was 70 mg/L to 440 mg/L in mud.
- **Industrial effluents:** A huge amount of pesticides are escaped by pesticide producing industries.
- **Domestic sewage:** DDT level in Yamuna water has risen from 0.25 ppb to 0.558 ppb after the Najafgarh drains sewage into this river at Wazirabad in Delhi.

Other sources include, suspended particulates, accidental spillage, evaporation from soils and plants, forest cover, residues in horticultural products.

4.8.3 Harmful Effects of Pesticides

Pesticides have the various types of effects on different living beings.

Effects on humans

Pesticides have the following effects on humans:

- Higher concentration of DDT in body parts and blood of human beings causes anxiety, tension, cancer, mutations, stress reactions, congenital and impotency.
- Central nervous system is the target of DDT poisoning in man which ultimately leads to death. It dissolves in lipids and accumulates in the body facts (3 to 90 ppm).
- The most threatening fact is DDT concentrates and accumulates in the food chain. It is continuously recycled in living- organisms. The level of DDT estimated in human tissues lie, in range of 5 to 10 ppm. According to an estimation DDT concentration in man's body fat varies from 3.3 g/m^3 in UK to 25 g/m^3 in India.
- Low levels of DDT cause cancer, high blood pressure and cirrhosis of the liver. DDT also interferes with signal transmission at the synoptic gap.
- Insecticides like BHC, aldrin, dieldrin, chlordane and endosulfan are extremely toxic to living beings. These are considered to affect the vital organs, heart, brain, kidneys and liver producing chronic disturbances.
- Pesticide 2, 4, 5-T consists of highly toxic component called dioxin or TCDD which is teratogen and liable to cause congenital malformations.

- Organophosphates like ethion, fonthion, dimethoate, trithion, monocrotophos, diazinon, dursban, phosdrin, thionazin and metasystox inhibit the production of cholinesterase at the junction between adjoining nerve, cell with the result that cholinesterase breaks down acetylcholine secreted by nerve cell axon. Excessive accumulation of acetycholine interferes with the nerve impulse transmission.
- The organo phosphorus insecticides such as parathion, malathion and TEPP may be absorbed by the lungs, eye membrane and skin to toxic amounts. This excessive absorption leads to larger accumulation of acetylcholine in the body disturbing the normal functioning of blood.
- Chronic accumulation of pesticides plays a major role in liver and kidney malfunctioning, secretion of excess of amino acids in human blood and urine, blood abnormalities as well as electroencephalogram deformations of brain tissues.
- Today children born have to start their life with a body burden of pesticides with increases with age. Pesticides are also reported to cause chronic impotency in man.
- On Dec, 3, 1984 Bhopal industrial disaster was the worst ever pesticidal MIC accident in history, taking an unprecedented and still unaccounted death toll leaving no fewer than 50,000 quickly dwarfed by the tidal wave of man's suffering that spread across the central city like the poison cloud. This carbaryl (carbamate pesticide) leakage caused an increased risk of sleeping, digestive problems, vision problems and sterility.

Effects on animals

Pesticides have the following effects on animals:

- Insecticides such as aldrin, dieldrin, chlordane, endosulphan, heptachlor and gammexane are reported to affect the wild life by changing their metabolic activities and body chemistry.
- Animals which have become weak and moribund as a result of exposure to pesticides may easily be destroyed by predators.
- Chlorophenoxy acid compounds such as 2, 4-D affect embryos while triazones may cause mutagenic effects in animals.
- Organophosphate pesticides cause extreme muscular weakness, tremors and dizziness in poisoned animals.

Effects on birds

Pesticides have the following effects on birds:

- Pesticides such as parathion, malathion, aldrin, dieldrin, heptachlor and several other organochlorines are reported to affect severely the metabolism in birds. Many species of hunting birds, particularly those having high levels of DDT are threatened with extinction.
- DDT has been implicated as the main villain and the cause of thin and fragile egg shells because of inhibition of enzymes and interference with the hormones that control calcium metabolism.

NOTES

- Pesticide pollution reduces egg laying in birds and fewer youngs are hatched.

Effects on aquatic biota

Pesticides have the following effects on aquatic life:

- Aquatic animals are extra ordinarily sensitive even to low concentrations of DDT. Even one part of DDT per trillion seems to cause death in brine shrimps.
- Acute danger arises from DDT which results in changing the behaviour and metabolic activities in sea animals.
- It is reported that marine invertebrates can concentrate pesticides by a concentration factor upto 70000 or more.
- Experiments have shown that DDT causes depression of photosynthesis in planktons. Its very low concentration as 1 ppm has immobilizing effects on Simocephalus species.
- Endrin is extremely toxic to fishes like blue gills and rainbow trout with LC_{50} values being 0.006 and 0.007 ppm respectively, while DDT is much less toxic to these fishes with LC_{50} values being 0.016 to 0.018 ppm respectively.
- Even the raptorial hawks and falcons are severely affected by DDT which causes breeding failure and death in them.
- Recent studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms, kill eggs and larvae of clams and oysters, influence the behaviour of fishes such as schooling and feeding and deteriorate water quality.
- Pesticides induce changes in blood chemistry and enzymatic functions of marine invertebrates. They reduce their backbonic collagen content and indirectly interfere with food chain.
- Since slight concentrations of organochlorine pesticides affect reproduction in fishes, there is every possibility that these pesticidal pollutants may adversely affect local fishery.
- Varieties of crustacea that make up the most valuable marine harvest are badly affected by pesticides. The oysters might be susceptible because of their tendency to concentrate and store trace chemicals from the surrounding environment.
- Pesticides-induced mortality pattern of marine molluscs, crustaceans and teleosts are also measurably related to various physico-chemical environmental parameters like concentration of pesticide and duration of its exposure.
- PCBs are reported to be concentrated in the food chain of marine ecosystems.
- Pesticidal pollutants ranked second to all other industrial wastes.

NOTES

Effect on grains

Many vegetables, fruits, rice, cereals and grains such as wheat, maize, grams and barley have been found to be contaminated with significant amounts of DDT and BHC. The level of DDT residue vary from 1.6 to 17.4 ppm in wheat, 0.8 to 16.4 ppm in rice, 3 to 17 ppm in pulses, 3 to 19 ppm in ground nuts, upto 5 ppm in vegetables and 68.5 ppm in potatoes.

Effects on soil

Pesticides not only pose potential hazards to man, animal, livestock, wild life and fish but they seriously interfere with the desired use of soil and water as follows :

- The most dangerous characteristic of pesticides, particularly organochlorines, is their long persistence in the soil which causes numerous adverse effects on grain quality.
- Even the accepted dosages of pesticides create deleterious effect in the long run in the soil.
- Pesticide retained in soil also concentrate in some crops, vegetables, cereals and fruits which taint them to such an extent that they are not usable.
- American Scientists observed that raddish and carrots grown on a loamy soil treated with aldrin at o one lb/acre contained 0.03 and 0.05 ppm of it respectively.
- Retention of pesticides in bottom soil of ponds renders them unsuitable for fish culture.
- Soil contaminated by pesticides also causes pollution of water sources, water supply and ground or surface water by rain.

4.8.4 Pesticide Analysis

Various methods employed widely for the analysis of pesticides, their residues and degradation products include:

(i) Chromatographic methods: These methods include TLC, GLC and HPCL. Organochlorides and carbamates are best analysed by TLC. Organophosphates are accurately detected by GLC using flame ionisation detector.

Pesticide analysis by this layer Chromatography (TLC)

This layer chromatography (TLC) is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic or glass which is coated with a thin layer of adsorbent material. On the completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (RF) expressed as:

$$R_f = \frac{\text{distance travelled by sample}}{\text{distance travelled by solvent}}$$

TLC is one of the fastest and least expensive, simplest and easiest chromatographic technique. Applications of this technique for the analysis of pesticides are discussed below:

NOTES

1. Analysis of organochlorine pesticides: Walker and Beroza separated 62 pesticides in 19 solvent systems by TLC. They employed silica geIG layer for chromatographing DDT, BHC, aldrins endrins. dieldrins, isodrin, chlordane and pentachlorophenol.

- Petroleum ether was used to separate DDT and four isomers of BHC.
- Cyclohexane was used to separate aldrin from its stereoisomer isodrin and dieldrin from its stereoisomer endrin,

Detection

Aidrin and its isomers were detected spraying with 0.01 N $KMnO_4$ solution. DDT and BHC were made visible by spraying with monoethanolamine and heating to 100°C for 20 minutes. This was followed by a spray of 0.1 N $AgNO_3$—HNO_3 (10 :1). The sprayed plates were exposed to UV lamp for one minute. Violet to green spots were observed.

R_f values of organochlorine pesticides when the chromatoplates are sprayed with 0.5% solution of *p*-dimethylamino hydrochloride prepared in sodium ethoxide are:

Pesticide	DDT	BHC	Aldrin	Dieldrin	Methoxychlor
R_f Value	0.59	0.39	0.78	0.18	0.12

2. Analysis of organophosphorus by TLC: Organophosphates can be chromatographed on silica gel G using hexane: acetone (4 : 1) as the solvent system. The chromatophates are sprayed with 0.5% solution of $PdCl_2$ in HCl giving yellow spots.

Pesticide	Parathion	Malathion	Chlorthion	Diazinon	Metasystox
R_f Value	0.66	0.52	0.44	0.78	0.62

Pesticide analysis by gas chromatography (GC)

In gas chromatography (GC) technique, the components of a sample are dissolved in a solvent and vaporized in order to separate the analysts by distributing the sample between two phases a stationary phase and a mobile phase. The mobile phase is a chemically insert gas that serves the heated column. The stationary phase is either a solid adsorbent, termed as Gas solid chromatography (GSC), or a liquid on an insert support, termed as gas-liquid chromatography (GLC). Analysis of organochlorine pesticide by gas chromatography is discussed below.

The pesticidal sample is extracted repeatedly with 15% ethyl ether in hexane. The ether extracts are evaporated on a steam bath to 2ml and diluted with hexane to 5 mL. A aliquot of this extract is injected into the gas chromatographic column (180ºC) with a microsyringe using Ar/CH_4 as the carrier has at 70 mL minute. The pesticides are vapourised. They move through the column at different rates and detected by electron capture detector.

NOTES

Organophosphorus pesticides can be analysed by GC using dichloromethane as the extractant and flame ionisation detector. Phenoxy herbicides (2,4-D and 2, 4, 5-12) can be analysed by derivatisation GC using electron capture detector. The herbicides are extracted with ether and after hydrolytic precipitation, methyl ester is analysed by gas chromatography.

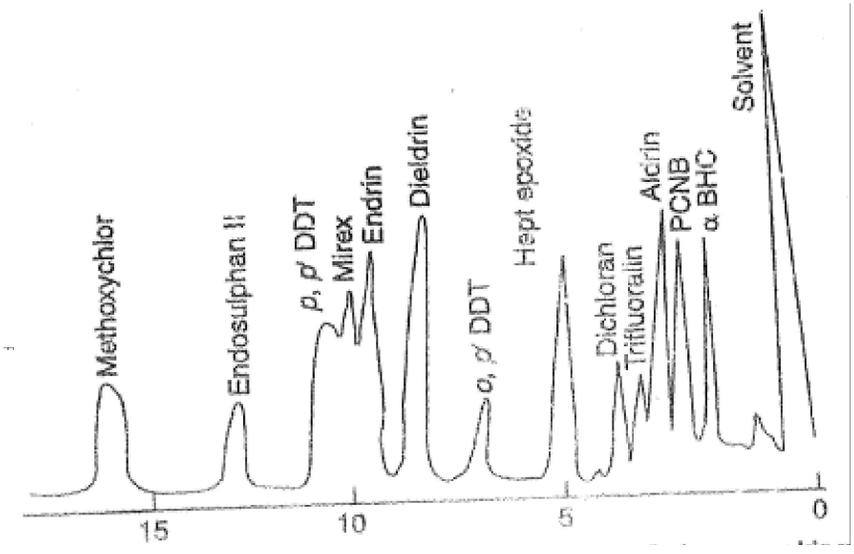


Fig. 4.2 *Gas Chromatogram of Organochlorine Pesticides. Columm packing 5% OV. Glass Column 180 cm × 4 mm i.d. Solid support Gas-chrom Q (100/128 mesh)*

Pesticide analysis by high performance liquid chromatography (HPLC)

HPLC is an analytical technique to separate, identify, and quantify components in a mixture. HPLC is highly improved form of column chromatography. A pump forces a solvent through a column under high pressures of upto 400 atm. The column packing material or adsorbent or stationary phase is typically a granular material made of solid particles such as silica or polymers. The pressure makes this technique much faster than column chromatography. The pressured liquid is typically a mixture of solvents such as water, acetonitrile and/ or methanol is referred to as mobile phase. The components of mixture are separated from each other due to their different degrees of interaction with the adsorbent pentacles. This causes different elution rates for the different components and leads to the separation of the components as they flow out the column. Compared to column chromatography, HPLC is highly automated and sensitive.

Analysis of insecticides, fungicides and herbicides by HPLC is discussed below.

(ii) Pesticide analysis by polarography and spectrometric methods

Organochlorine pesticides such as aldrin, endrin and BHC can be analysed on DME in LiCl in isopropanol. The supporting electrolytes are 0.1 M HNO_3, acetate buffer, NH_3——NH_4Cl buffer and 0.1 M NaOH. The half wave potential ($E_{1/2}$) in these electrolytes varies from –0.32 V to 0.72 V. In case of parathion, the $E_{1/2}$ is –0,26 V, –0.62 V and +0.74 V in 0.1 M HNO_3, acetate buffer and NH_3–NH_4Cl buffer respectively.

NOTES

In spectrophotometric methods methods, the standardised adsorbents are generally required for clean up before analysis, JR. and UV methods have been used for the analysis of various isomers of BHC. NMR helps to identify metabolites and degradation products of pesticides. Carbamates, chlorinated insecticides and their metabolites can be analysed by mass spectrometry. PCBs, lindane and 2, 4, 7, 8-T have successfully been analysed by GC-MS technique.

4.9 WATER QUALITY LAWS AND STANDARDS

Water is used for various purposes for daily life, agriculture, industry and fisheries water in chemically pure form rarely occurs in nature. It is found to host a wide variety of constituents derived from both the natural and human environments. It is therefore necessary to establish water quality standards to assess and to manage water from viewpoint of water as well as the resource availability. Water quality standards must be developed from scientific evidence that of adverse effects on ecological system including human beings and their water usage. The water quality standards in India are based on water quality standards in the stream and specify its suitability for different purposes such as drinking, irrigation, industry, public health and environmental safety. Indian Standard Institution (ISI) is responsible in specifying the norms for various effluents so that the ambient water quality standards are maintained.

The different water quality standards in India are enacted under following categories in India. Specific laws have been enacted by both the state governments and central Government in India to control water pollution. All these laws are based a water funders, as set of parameters used to define water quantity.

1. **Central enactments:** Acts passed by Government of India are:
   (i) North India Canal and Drainage Act, 1873.
   (ii) Indian Fisheries Act, 1897.
   (iii) Damodar Valley Corporation Regulation Act, 1948.
   (iv) The River Boards Act, 1956
   (v) The Water (Prevention and Control of Pollution) Act, 1974 (Amended in 1988)
   (vi) The Environment (Protection) Act, 1986
   (vii) The Merchant Shipping Act, 19872.
2. **State enactment:** Acts passed by State Governments are:
   (i) The Orissa River Pollution Prevention Act, 1953,
   (ii) The Maharashtra Prevention of Water Pollution Act, 1969.

Let us study the two major Acts in detail.

- **The Water (Prevention and Control of Pollution) Act, 1974 Amended in 1988):** The Act defined terms like pollution, sewage effluent, trade effluent, stream and boards. The Act also assigns the functions to be carried out by the Central and State Boards. The Water Boards have power to obtain

information, to take samples of effluents from any industry and to make survey of any area and gauge and keep record of the volume and other characteristics of any stream or well. A person empowered by the Board has the right to enter, inspect and examine any plant, record register, document or any other material object, or for conducting a search of any place where he has reason to believe that no offence of water pollution is committed. The Board has wide powers to prohibit the use of any stream or well for discharging any pollutant in it. The Board has powers to restructure the outlets for dumping pollutants,

The Act prohibits disposal of any poisonous, noxious or polluting matter to the flow of water in a stream. However dumping or any material into a stream for the purpose of reclamation of land is not considered an offence, The Act provides for severe and deterrent punishments for violation of the Act which includes fine and imprisonment.

- **Water (Prevention and Control of Pollution) Cess Act, 1977 (Amended in 1991):** The Act empowers the Central Water Board to collect cess on water consumed by persons carrying on certain scheduled industries and by local Authorities responsible for supplying water. The cess and the consent fees form the major sources of revenue to run the Central and State Water Boards. The Act has been amended in 1991 with a view to augment the resources of the Boards by removing the lacunae in the Act and to provide rebate to the industries for complying with the consumption and effluent quality standard.

Technical difficulties in controlling water pollution

There are, however, several enforcement problems. Although the Water Cess Act was passed to meet the expenses of the Central and State Boards yet Water Board has no power to take direct action against the erring party, The court procedures are time consuming and delays often prevent quick and preventive action thereby defeating the sole purpose of the Act. Because of the problems inherent in the implementation of the Act, amendments were proposed for strengthening the working of the State Boards. Inspite of the legislative measures, the pollution of our water ways continues unabated. This is due to the lack of civic sense among people and due to the lack of necessary infra structure for enforcing implementation of the laws efficiently.

Some of the water standards are as follows:

1. Drinking water standards: National and international authorities have laid down various standards for domestic use of drinking water. The main agencies include IST, Indian Council of Medical Research (ICMR), Ministry of Works and Housing (MWM, World Health Organisation (WHO), United States Public Health Standards (USPHS) etc. Parameters for water quality characterisation and standards are illustrated in Table 4.4.

NOTES

Table 4.4 *Drinking Water Standards (Domestic Water Supplies)*

Parameter	USPHS	ISI	Parameter	USPHS	ISI
Colour Odour Taste	Colourless Odourless Tasteless	—	pH	7.0-8·5	6.0–9.0
Specific conductance	300 mmho cm^{-1}	—	Turbidity	25 JTU	25 JTU
Suspended solids	5.0	——	Total dissolved solids	500	—
Chloride	250	600	Sulphate	250	1000
Cyanide	0.05	0.01	Nitrate + nitrite	<10	—
Fluoride	1.5	3.0	Phosphate	0.1	—
Sulphide	0.1 mgL^{-1} (ppb)	—	Ammonia	0.5	—
Boron	1.0	—	Calcium	100	—
Magnesium	30	—	Arsenic	0.05	0.2
Barium	1.0	—	Cadmium	0.01	—
Chromium VI	0.05	0.05	Copper	1.0	—
Iron	<0.3	—	Lead	<0.05	0.01
Manganese	0.05	—	Mercury	0.001	—
Selenium	0.01	—	Silver	0.05	—
Zinc	5.5	—	COD	4.0	—
Carbon $CHCl_3$ extract	0.15	—	Phenols	0.001	0.005
Pesticides (total)	0.15	—	PAH	0.2 ppb	—
Surfactants	200	—	Dissolved oxygen	4.0–6.0 ppm	—
Gross beta radioactivity	1000 pc/L	—	Radium-226	3pc/L	<5000
Strontium 90	10 pc/L	<5000	Coliform cells/100 mL	100	—
Total bacteria count/ 100 mL	1×10^6	—	Detergents	0.1	—

Note: All the units, except otherwise mentioned, are in ppm or mg/L.

2. Stream standards: The permissible limits of drinking water parameters are 3 to 5 times higher for surface waters. The water quality criteria are classified into different categories by CPCB and SPCB depending on the use of water for irrigation, drinking, industry, power generation and recreation.

Table 4.5 *Classification of Waterbodies by CPCB.*

Class	Use of Water
A	Drinking water source without conventional treatment but after disin
B	Outdoor bathing.
C	Drinking water source with conventional treatment followed by disin
D	Propagation of wild life, fisheries, irrigation.
I	Sea water including estuaries and coastal waters.
II	Shell fishing, contact sport.
III	Commercial fishing, non-contact recreation.
IV	Industrial cooling, navigation and controlled waste disposal

There should be no visible discharge of domestic and industrial wastes into class A waters. In case of class B and C, the discharges shall be regulated so as to ensure the maintenance of the stream standards.

NOTES

3. Irrigation standards: The major parameters of concern are:

(i) Salinity: Salinity or total dissolved solids is the most important parameter for irrigation water since it controls the availability of water to plants through osmotic pressure regulating mechanisms.

(ii) Water infiltration rate: High sodium and low calcium content of water reduces the water infiltration rate so that sufficient water can not reach the crop roots.

Table 4.6 *Water Quality Criteria for Freshwater Classification by CPCS*

Class	Water quality criteria
A	DO (6 mg/L), BOD (2 mg/L), MPN of conforms per 100 mL (maximum 50), pH (6.5–8.5)
B	DO (5 mg/L), BOD (3 mg/L), MPN of coliforms per 100 mL (maximum 500), pH (6.5–8.5)
C	DO (4 mg/L), BOD (3 mg/L), MPN of conforms per 1.00 mg/L, (maximum 5000), pH (6.0-9-0)
D	DO (4 mg/L), pH (6.5-8.5), Free NH_3 as N (1.2 mg/L)
E	pH (6.0—8.5), conductivity (2,250 mS/cm), sodium absorption ratio (26), boron (2 mg/L)

(iii) Specific ion-toxicity: The presence of toxic metals such as Cu, Cd, As, Pb, Hg, Mn, Ni etc. in water cause crop damage and reduce agricultural yield. The tolerance limits for trace metals (in ppm) in irrigation water are:

Al	As	Be	B	Cr	Co	Cu	Pb	Li	Mn	Ni	Zn	V
1.0	1.0	0.5	0.75	5.0	0.2	0.2	5.0	5.0	2.0	0.5	5.0	10.0

(iv) Sodium concentration: Sodium concentration in water can be denoted by

- Percent sodium $= \dfrac{Na^+}{Ca^{2+} + Mg^{2+} + K^+ + Na^+} \times 100$

- Sodium absorption ratio (SAR) $= \sqrt{\dfrac{Na^+}{Ca^{2+} + Mg^{2+}/2}}$

- Residual sodium carbonate $= \left(CO_3^{2-} + HCO_3^-\right) - \left(Ca^{2+} + Mg^{2+}\right)$

The values of individual constituents are taken in mg/L.

Suitability of water for irrigation with different value of sodium absorption ratio (SAR) is illustrated below:

- **SAR, 0-10:** Suitable for all types of soils and crops.
- **SAR, 10-18:** Suitable for coarse textured or organic soil with good permeability. Unsuitable for fine soil.

NOTES

- **SAR, 18-26:** Harmful for all types of soils, requires good drainage, high leaching and gypsum addition.
- **SAR, > 26:** Unsuitable for irrigation purposes

(v) Miscellaneous constituents: Excess of nitrate ions in water causes eutrophication which is injurious for the plant growth.

4. Bacteriological standards: These standards are set by WHO and Ministry of workds and Housing.

(i) E. coliform bacteria count in any sample of 100 mL water should be zero.

(ii) Total bacterial count not more than 10/100 mL should be resent in any sample.

5. Effluent standards: Effluent standards are related to the quality of waste waters originated from industry, community and agriculture. They are shown in Table 4.7.

***Table 4.7** Effluent Standards*

Parameter	Standards			
	In land surface water	**Public sewers**	**Land for irrigation**	**Marine coastal area**
pH	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0
Suspended solids	100	600	200	100
Dissolved solids	2100	2100	2100	—
BOD (5 days at 20°	30	350	100	100
Oil and grease	10	20	10	20
Total residual chlorine	1.0	—	—	1.0
Sulphate	1000	1000	1000	—
Total N	100	—	—	100
NH3–N	50	50	—	50
Chloride	1000	1000	600	—
Fluoride	2.0	15	—	15
Fercent Na	—	60	60	—
Phenolic compounds	1.0	5.0	—	5.0
Alpha emitters μC/mL	10^{-7}	10^{-7}	10^{-7}	10^{-7}
Beta emitters μC/mL	10^{-6}	10^{-6}	10^{-6}	10^{-6}

Note. Maximum limits of parameters are expressed in ppm or mg/L

Check Your Progress

12. List the uses of manganese.
13. Define dissolved oxygen.
14. What is determined by the COD test?
15. Write the precautions taken while performing BOD test.
16. How do pesticides reach soil?
17. What is the use of thin layer chromatography (TLC)?

4.10 ANSWERS TO 'CHECK YOUR PROGRESS'

NOTES

1. Wastewater is water whose physical, chemical or biological properties have been changed as a result of introduction of certain substances which render it unsafe for some purposes such as drinking.
2. The major sources of waste water are domestic sewage, industrial waste water, agricultural soil and radioactive wastes.
3. In case of a heterogeneous liquid like sewage, a composite sample is taken.
4. Although standard method but Jackson model permits measurement of turbidity from 25 to 1000 JU.
5. Conductivity of water varies directly with the temperature and is proportional to its dissolved mineral matter content.
6. The term 'solid' refers to the matter that remains as residue upon evaporation. Total solid includes both dissolved solids and suspended solids.
7. Acidity is a measure of the effects of combination of compounds and conditions in water.
8. Heavy metals are defined as metallic elements that have a relatively high density compared to water.
9. Copper is necessary for the normal biological activities of amine oxidase and tyrosinase enzymes.
10. Cadmium toxicity causes anaemia, hypertension, adrenal dysfunction, bone marrow disorders, cancer, damage to kidney lungs and liver.
11. Chromium is distributed in the earth crust at about 200 ppm levels and in sea water at 3 ppb.
12. Manganese is used in dry battery cells, electrical coils, ceramics, alloys and in glass and steel manufacturing industries.
13. Dissolved oxygen refers to the level of free non-compound oxygen resent in water or other liquids.
14. COD test is also used to determine the amount of inorganic chemicals in a sample.
15. The precautions while performing the BOD test are as follows:
    - The temperature of 20°C shoould be constantly maintain during the 5 days incubation period. Even an increase or decrease of temperature by 1°C may increase or decrease the BOD value by about 4.7%.
    - The BOD test should be performed as soon as possible after the sample collection.
16. Pesticides contaminate in vapour phase in air, get adsorbed onto the dust particles which ultimately reach soil or water along with the rain water.
17. Thin layer chromatography (TLC) is a technique used to isolate non-volatile mixtures.

NOTES

4.11 SUMMARY

- The day-to-day activities of man is mainly water dependent and therefore discharge 'waste water' into water.
- The major sources of waste water are domestic sewage, industrial waste water, agricultural soil and radioactive wastes.
- The analysis of water pollutants is extremely important as it contains a large number of impurities which are necessary to be checked before the water is used for any specific purpose.
- Sampling sewage involves the collection of grab samples in a bottle of capacity 100 in L and combining them in a single container after 24 hour period.
- Preservation of water pollutants is essential to protect samples from changes in composition, deterioration, retarding biological action, reduction of volatility of constituents.
- There are various parameters for water analysis are colour, turbidity, conductivity, total solids, acidity, alkalinity, hardness, chloride, sulphate, fluoride, silica, phosphate and different forms of nitrogen.
- Heavy metals are defined as metallic elements that have a relatively high density compared to water.
- Chloride in drinking water is harmless if present below 250 ppm but its higher content harms metallic pipes and crops.
- Sulphate usually occurs in natural waters. Mine drainage wastes also contain high content of sulphate by virtue of pyrite oxidation.
- Fluoride occurs is all natural water supplies and in chemical wastes from industries.
- Fluorides if present in small quantities upto 1 ppm, are generally considered as beneficial.
- Silica in water is not a pollutant, but its higher concentration in water is undesirable.
- Industrial effluents, domestic sewage and agricultural run offs are the major contributors of phosphates in water.
- Nitrogen occurs in water in small amounts and in bound form it is found as NH3, NO^{3-}, NO^{2-} and organic nitrogen.
- Heavy metals are defined as metallic elements that have a relatively high density compared to water.
- Due to high degree to toxicity, arsenic, cadmium, chromium, lead, mercury are the priority metals that are of public health significance.
- The various methods employed for the nonitoring of metal pollutants from the atmosphere and aqueous sources include atomic absorption spectrophotometer (AAS), atomic emission spectroscopy (SAS), inductively coupled plasma atomic emission spectroscopy (1CP-AES), ion

selective electrodes, fluorescene, plasma spectroscopy, mention activation analysis (NAA), thermogravimetric analysis (TGA), etc.

- Dissolved oxygen refers to the level of free non-compound oxygen resent in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a water body.
- Chemical oxygen demand (COD) is the amount of oxygen needed to oxidize the organic matter present in water.
- Biochemical oxygen demand (BOD) is the amount of dissolved oxygen (DO) needed by aerobic biological organism to break down organic material present is a given water time period.
- The pesticide is a composite term that includes all chemical that are used to mill orcontrol pests. In agriculture, this includes herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (vertebrate poisons).
- The water quality standards in India are based on water quality standards in the stream and specify its suitability for different purposes such as drinking, irrigation, industry, public health and environmental safety.
- The Water (Prevention and Control of Pollution) Act, 1974 was amended in I988). The Act defined terms like pollution, sewage effluent, trade effluent, stream and boards.

4.12 KEY TERMS

- **Wastewater:** It is water whose physical, chemical or biological properties have been changed due to introduction of certain substances.
- **Water Pollution:** It refers to the contamination of water bodies, usually as a result of human activity, in such a way that their lawful uses are harmed.
- **Heavy Metals:** These are metallic elements that have a relatively high density compared to water.
- **Pesticides:** These substances are used to control pests.
- **Dissolved Oxygen:** It refers to the level of free non-compound oxygen resent in water or other liquids.
- **Chemical Oxygen Demand:** It refers to the amount of oxygen needed to oxidize the organic matter present in water

4.13 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Mention the objectives of analysis of water quality.
2. How is a sampling of industrial effluents and wastewater done?
3. Differentiate between suspended solids and dissolved solids.

NOTES

4. What is electrical conductivity? How is it measured?
5. What are the factors responsible for heavy metals toxicity?
6. What are the apparatus and reagents used in atomic absorption spectrophotometry for heavy metal analysis?

Long-Answer Questions

1. Discuss the origin of wastewater.
2. Analyse the various parameters for water analysis.
3. Give the principle and method to determine alkalinity using tetrimetric method complexometric titration.
4. Discuss the occurrence and industrial uses and pollution sources of cadmium. Also, mention its harmful effects on human health.
5. Describe the toxic effects of pesticides on different living creatures.
6. Explain pesticide analysis by gas chromatography in detail.
7. Discuss various water pollution laws and standards in detail.

4.14 FURTHER READING

Dash, Dhruba Charan. 2011. *Analytical Chemistry*. New Delhi: Prentice Hall India Pvt, Limited

Hibbert, D.B. and J.J. Gooding. 2006. *Data Analysis for Chemistry*. UK: Oxford University Press.

Benwart, G. J. 1987. *Basic Food Microbiology*. New Delhi: CBS Publishers & Distributors.

Christian, Gary D. 2007. *Analytical Chemistry*, 6th Edition. Noida: Wiley India Pvt. Limited

UNIT 5 ANALYSIS OF SOIL, FUEL, BODY FLUIDS AND DRUGS

NOTES

Structure

5.0 INTRODUCTION

Analytical chemistry is focused on improvements in experimental design, chemometrics, and the creation of new measurement tools. Analytical chemistry has broad applications to medicine, science, and engineering. Soil analysis is a set of various chemical processes that determine the amount of available plant nutrients in the soil, and also the chemical, physical and biological soil properties important

NOTES

for plant nutrition, or "soil health". Chemical soil analysis determines the content of basic plant nutrients; nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), pH, humus content, total $CaCO_3$, available lime, organic matter, total Sulphur (S), trace elements, and other physical characteristics (capacity, permeability, density, pH - value).

It is important to have knowledge about fuel quality in order to be prepared to handle operating problems such as slag formation or corrosion. From optimizing feedstock selection to determining price, fuel analysis is critical to the oil and gas industry. The molecular structures of crude are complex and can vary significantly. This makes fuel analysis an important part of the upstream, midstream and downstream stages. The analysis of body fluids plays a critical role in the diagnosis and prognosis of a disease. A change in concentration or composition of a particular biochemical constituent in body fluids is used as an indicator of a physiological or pathological condition. Clinical chemistry is the area of chemistry that is generally concerned with analysis of bodily fluids for diagnostic and therapeutic purposes. It is an applied form of biochemistry. Drug analysis includes the aspects of identifying novel drugs, assessing their affinity and specificity, characterizing their molecular structures, and testing their efficiency in vitro and in vivo. Drug analysis encompasses tests on raw materials (purity criteria), pharmaceutical or veterinary formulations, and a number of other, more complex matrices such as foods of animal origin, drinks, and foodstuffs that are conducted for clinical, forensic, or veterinary purposes involving a variety of matrices including blood, urine, and tissues. This unit will explain soil and fuel and their components. In addition, it will discuss the concept of clinical chemistry and immunoassay. Also, it will describe blood gas analysis and drug analysis in detail.

5.1 OBJECTIVES

After going through this unit you will be able to:

- Analyse soil and fuel and their components
- Understand the concept of clinical chemistry
- Explain immunoassay and its principles
- Evaluate blood gas analysis
- Discuss the concept of drug analysis

5.2 ANALYSIS OF SOIL

The word '*Soil*' is derived from the latin word '*Solum*' meaning ground. Here, the word soil is used in a strictly scientific sense. To the scientist soil is not merely the product of simple rock decay - the result of both chemical decomposition and mechanical disintegration of rocks, whether igneous or sedimentary, but a reworked natural material having special and definite characteristics of its own.

Soil is defined as a thin layer of earth's crust which serves as a natural medium for the growth of plants. Soil is formed by weathering process of parental material rocks. Soil differ from parental material in the morphological, physical,

chemical and biological properties. Generally it is said that, it is a mixture of mineral constituents and of organic matter, which later consists, more or less of the decomposed residues of plant materials and to a lesser extent of animal remains and extreta.

There are various types of soils like red, black, yellow - alluvial, etc. Depth of soil is variable and it differ in texture like coarse, fine, soils serve as reservoirs of nutrients and soil's flora. The most characteristic constituents of soil are the colloidal organic matter and colloidal clay. Soil organic matter and particles conserve soil moisture for crops, plantation, soil living organisms. Soil provide even mechanical anchorage and favourable tilth. The components of the soil are mineral material, organic material,water and air the proportion of which vary.

Since, the main interest of soils to MAN is for growing his crops, it is necessary to learn also about the physical and chemical properties of the soils. Because for growing with success particular crop is dependent. In soil studies, the following physical properties are important

(*i*) Specific gravity (*ii*) Pore space (*iii*) Plasticity

(*iv*) Cohesion (*v*) Colour (*vi*) Texture

(*vii*) Soil temperature (*viii*) Permeability.

Rocks are the chief sources for the parent materials over which soils are developed. There are three main kind of rocks viz (*a*) igneous rocks (*b*) sedimentary rocks (*c*) metamorphic rocks. Soil differs in chemical composition. The main chemicals are SiO_2, Al_2O_3, Fe_2O_3, MgO, FeO, CaO, Na_2O, K_2O, CO''_3, P_2O_5, MnO, TiO_3 etc. and water moisture. Weathering, soil formation and development proceed simultaneously. The weathering may be chemical or physical. Physical weathering comprised of — water, temperature, wind, plants/animals, while chemical weathering comprised of solution, hydration, hydrolysis, carbonation, oxidation-reduction.

5.2.1 Soil Composition: Moisture, pH, Total Nitrogen, Phosphorus, Silica, Lime, Magnesia, Maganese, Sulpher and Alkali Salts

Soil comprises a mixture of inorganic and organic matter with water and air in invariable proportions. The components are as follows:

• Inorganic matter (45%)

Soil is a complex mixture of various inorganic compounds. The main elements are silicon, aluminum, magnesium, calcium, potassium, sodium and iron. Traces of elements like boron, manganese, copper, iodine are also present in the soil. The approximate weight percent composition of the elements is:

- Oxygen – 46.6 per cent
- Silicon – 27.7 per cent
- Aluminium – 8.1 per cent
- Iron – 5.0 per cent
- Calcium – 3.6 per cent
- Sodium – 2.8 per cent
- Potassium – 2.6 per cent
- Magnesium – 2.17 per cent

NOTES

The compounds on the basis of anions are:

- Silicates of Na, K, Ca, Al and Fe, the soil is essentially silicate mineral. Approximately, 75 per cent of the inorganic materials are silicates. Finely divided SiO_2 (Quartz) is commonly found in soil.
- The most commonly available silicates in soil are orthoclase ($KAlSi_3O_8$), albite ($NaAlSi_3O_8$) and epidote ($4CaO_3\ (AlFe)_2O_3.\ 6SiO_2.\ H_2O$).
- Oxides of Fe, Mn such as magnetite Fe_3O_4, sulphates, chlorides and nitrates.
- Carbonates, sulphates, chlorides, nitrates of Ca, Mg, Na and K, etc. like $CaCO_3$, $MgCO_3$, $CaSO_4$, $CaCl_2$, $Ca(NO_3)_2$.

Clay is essentially a mixture of hydrated aluminum and iron silicates which bind cations such as Na^+, K^+, Ca^{2+}, Mg^{2+} and NH_4^+. Due to such binding, the cations are not leached easily and remain for a longer duration and are available as plant nutrient.

• Organic matter (5%)

The soil productivity is determined primarily by organic matter and usually it constitutes less than 5 per cent of the soil. The organic matter consists of biologically active compounds like polysaccharides, nucleotides, organophosphate, organosulphur compounds and humus. Partially decomposed organic matter in a soil is referred to as 'humus'. After the death of organisms, different substances like carbohydrate, proteins, fats, etc., are released into the environment and the rest is called 'humus'. There are billions of macro and microorganisms such as virus, bacteria, fungi (non-green plants), protozoa (amoeba, euglena, paramecium, entamoeba), etc., which live in the soil. The dead bodies and various parts of plants and animals (all considered as wastes) are decomposed by some of the above mentioned microorganisms known as decomposers and these living organisms that grow on dead or decaying organisms are known as 'saprotrophes'. These saprotrophes break down the complex organic matter, releasing minerals that get mixed up with soil and contribute to soil fertility. Some soil fungi yield citric acid and many other chelating organic acids which react with silicate minerals and liberate Na^+, K^+ or Ca^{2+} from the silicate matrix.

• Soil water (30%)

In soil, water is not only important as a solvent and a transporting agent, but it also maintains the soil texture, arrangement of soil particles and makes soil suitable for all living organisms. Water in soil mainly comes through infiltration of precipitated water (dew, rain, snow, hail) and irrigation. Soil water exists both in combined and uncombined state. Chemically combined water is the water of crystallization of mineral grains and water of hydration of clay mineral particles. Uncombined water is not chemically bound to soil particles and is held in soil by adhesion (adhere to soil particles through short range interaction) and cohesion (attraction between the water molecules). Adhesion or cohesion occurs in four different forms:

(a) Gravitational water: The accumulated excess water in large soil spaces, through displacement of air in course of downwardly movement of water under the gravitational influence, is known as gravitational water. When

gravitational water further percolates and reaches the level of parent rock, it is called ground water.

(b) Capillary water: Water which is held by capillary forces such as surface tension and cohesive forces of water molecules, which work when the gravitational and ground water is drained, is called capillary water.

(c) Hygroscopic water: Water molecules held tightly on the surface of soil particles is called hygroscopic water.

(d) Water vapour: Water present in the form of vapours or moisture in soil is known as water vapour.

The total waste in the soil is also called holard. The water that can be absorbed by plants from holard is called chresard and that cannot be absorbed is called echard. Thus,

Holard = Chresard +Echard

• Soil air (20%)

The air present in the spaces between soil particles is called soil air. The composition is usually O_2, N_2, CO_2 and H_2O (vapour). The soil air provides aeration of the soil, which in turn helps in absorption of water by plant roots. Soil air is also very important for oxidation of organic material in the soil, the absence of which leads to anaerobic activity.

Soils having pH below 7 are considered to be the acidic, but those which have pH less than 5.5 and which respond to liming may be considered to qualify to be designated as acid soils. It occurs widely in Himalayan region, Peninsula, Gangetic delta.

Check Your Progress

1. Define soil.
2. From which material is soil formed?
3. What is the approximate percentage of water in soil?
4. What is hygroscopic water?

5.3 FUEL ANALYSIS

When a substance is burnt in air or oxygen with the evolution of heat, the process is called combustion and the substance which is burnt is known as combustible substance. The heat evolved during a combustion can be used economically for industrial or other use. All combustible substances with contain carbon as the main constituent are called fuels. Thus, a fuel may be defined as, any combustible substance, chemical or reactant containing carbon as the main constituent which on proper burning produces heat that can be used economically for domestic and industrial purposes and in generation of power.

$$\text{Fuel} + O_2 \rightarrow \text{Products} + \text{Heat}$$

NOTES

5.3.1 Solid Fuels: Ultimate and Proximate Analysis and Heating Values

Solid fuels may be classified as natural or artificial. The natural fuels are wood, coal and other vegetable matter while the artificial solid fuels are coke and charcoal. Let us study them in detail.

(i) Wood: Wood is undoubtedly the first fuel used by man, and it is still used in large quantities especially in India, although in recent years its use as fuel in industry has been continuously decreasing, It is cheap and abundant especially in the tropical regions. Wood is dried vegetable tissues of trees, composed mainly of cellulose $(C_6H_{10}O_5)_n$, and contains nearly 60% of water. The principal use of wood is as domestic fuel, the soft resinous varieties such as spruce and pine being the most desirable. The chief advantages of wood as fuel are the ready inflammability, low ash content and fairly good flames, the only condition is that the wood should be dry. On burning, wood gives carbon dioxide and water.

$$(C_6H_{10}O_5)_n + 6O_2 \longrightarrow 6nCO_2 + 5n\ H_2O$$

The flame of burning wood may be coloured by impregnating the wood with nitrates of strontium (red flame), copper (green flame), calcium (brick-red flame), potassium (pale-violet flame), etc. The calorific value of air dried wood ranges from 3000 to 4000 kcal/kg.

The main drawbacks for using wood are its lower calorific value and high cost; of transporting at large distances by rail.

(ii) Coal: Coal is by far the most important solid fuel. It is formed by the action of decay, heat, and pressure upon vegetable and woody material deposited many years ago. Coal varies greatly in composition and properties, the most important types are peat, lignite, bituminous (soft) coal, and anthracite or hard coal. Following table gives approximate composition of various varieties.

Table 5.1 *Percentage Composition of Solid Fuels and their Calorific Values*

Type of cool	Carbon	Hydrogen	Oxygen	Ash	Calorific value (kcal/kg)
Wood	48.5	6.0	43.5	1.5	3000 – 4000
Peat	58.0	6.3	30.8	4.0	4000 – 5000
Lignite	68.0	5.1	19.5	6.3	4000 – 6000
Soft coal	82.0	5.0	6.0	7.0	6000 – 8000
Hard coal	90.0	2.5	2.5	4.0	8000 – 85000

Vast peat logs occur in the temperate humid climates of the world, formed by the accumulation and partial decomposition of vegetable matter under marshy conditions. It is mainly used as a fuel but only where there is a deficiency of coal. Its calorific value changes from 4000 to 5000 kcal/kg.

Lignite or brown coal is brown coloured intermediate stage between the peat and bituminous coal. Owning to a high ash content and very hygroscopic nature it is used only as local fuel. It burns readily with a firy long flame and has a calorific value changing from 4000 to 6000 kcal/kg . It is widely used for the production of producer gas. It is also used for steam raising in thermal plants for the manufacture of electricity.

Bituminous coal, soft coal or common coal is a compact black substance and is the most widely used of all the mineral coals. It is used for steam raising and for manufacturing coke.

Anthracite is much less abundant form of coal and contains but little volatile matter. It has a high calorific value, and is the most valuable form for industrial use. It is used for steam raising, for household purposes and as a metallurgical fuel.

(iii) **Coke:** It is obtained by the destructive distillation of coal. It burns with a small flame and without smoke. Its calorific value varies between 7000 to 8000 kcal/kg.

(iv) **Charcoal:** It is obtained by heating wood in limited quantity of air. It easily ignites and burns with a slight flame, without much smoke. Its calorific value varies between 6000 to 8000 kcal/kg. All the forms of coal as well as coke and charcoal burns to give off carbon monoxide and carbon dioxide.

$$2C + O_2 \longrightarrow 2CO \text{ (excess of carbon)}$$

$$C + O_2 \longrightarrow CO_2 \text{ (excess of carbon)}$$

Now let us study the analysis of cool in detail.

Coal, the most important solid fuel, is a mixture of various hydrocarbons formed by the combined action of high temperature and high pressure on vegetable matter over a geological period of time. Its composition varies widely and hence it is necessary, to analyze it and interpret the result for classification, price fixation and proper industrial utilization. The analysis of the coal is carried out in the following two ways:

(A) Proximate analysis: It includes the determination of moisture, volatile matter, ash and fixed carbon in a given sample of coal. It gives quick and valuable information regarding commercial classification and suitability for a particular industrial use. This method involves the following procedure:

(i) Determination of percentage of moisture: A known weight of coal is taken in a preweighed silica crucible and is maintained at 105–115°C for about an hour in an electrical oven. It is then taken out, cooled, and weighed. The process of heating, cooling, and weighing is repeated many times until the constant weight of the crucible is obtained. Knowing the loss in weight of the coal, percentage of moisture can be calculated as:

Let

Weight of empty crucible $= x$ g

Weight of crucible + coal sample $= y$ g

Weight of crucible + residue (coal without moisture) $= z$ g

Weight of the coal sample $= (y - x)$ g

Weight of the moisture $= (y - z)$ g

$$\text{Percentage of moisture} = \frac{\text{Weight of moisture}}{\text{Weight of coal}} \times 100 = \frac{(y-z)}{(y-x)}$$

NOTES

(ii) Determination of percentage of volatile matter: A known weight of moisture free coal is taken in a weighed crucible covered with the lid and is kept in a muffle furnace at 950°C ± 20°C for about 7 minutes. The hot crucible is taken out, cooled in a dessicator, and weighed again. The amount of weight loss is reported as volatile matter and is calculated as:

Let

Weight empty of crucible = x g

Weight of crucible + moisture free coal = y g

Weight of crucible + residue (coal without volatile matter) = z g

Weight of moisture free coal = $(y - x)$ g

Weight of volatile matter = $(y - z)$ g

$$\text{Percentage of volatile matter} = \frac{\text{Weight of volatile matter}}{\text{Weight of moisture free coal}} \times 100$$

$$= \frac{(y-z)}{(y-x)} \times 100$$

(iii) Determination of percentage of ash: A known weight of dry coal sample obtained in the above process is taken in a weight silica crucible. The crucible is then placed in an electric furnace maintained at 750° ± 20°C for about half an hour. The cruscible is taken out, cooled and weighed again. Heating, cooling and weighing is repeated till a constant weight is obtained. The percentage of ash is calculated as:

Let

Weight of empty crucible = x g

Weight of crucible + coal sample = y g

Weight of crucible + residue (ash) = z g

Weight of coal sample = $(y - x)$ g

Weight of ash = $(z - x)$ g

$$\therefore \text{ Percentage of ash} = \frac{\text{Weight of ash}}{\text{weight of coal sample}} \times 100 = \frac{(z-x)}{(y-x)} \times 100$$

(iv) Percentage of fixed carbon: The percentage of fixed carbon is determined as,

percentage of fixed carbon = 100% of (Moisture + volatile matter + Ash)

Significance of Proximate Analysis: The proximate analysis plays an important role in assessing the quality of coal in following ways:

- **Moisture:** Presence of moisture in the coal sample reduces its calorific value and a considerable amount of heat is wasted in evaporating the moisture during combustion. Hence, a high percentage of moisture is undesirable.
- **Volatile matter:** The presence of excess volatile matter lowers the calorific value of fuel, because in this case, the combustion will be

incomplete. Also high volatile matter containing coal gives long flames, high smoke and relatively low heating values. The volatile matter content is more in bituminous coal than in anthracite coals.

- **Ash:** Ash is a noncombustible matter and it reduces the calorific value of coal. It also cause hindrance in the flow of heat. It lowers the temperature and gives rise to clinker formation. Disposal of ash is also a big problem.
- **Fixed carbon:** The presence of fixed carbon increases the calorific value of the coal. It is this carbon that is burnt in the solid state.]

(B) Ultimate analysis: This analysis gives us information about the percentage of carbon, hydrogen, sulphur, nitrogen and oxygen present in the sample of coal. It involves the following determinations:

(i) Determination of percentage of carbon and hydrogen: A known weight of coal is taken in a combustion tube and is burnt in excess supply of oxygen. The CO_2 and H_2O formed are passed through two preweighed bulbs containing anhydrous calcium chloride and a second bulb containing potassium hydroxide. The increase in the weight of calcium chloride bulb represents the weight of carbon dioxide formed. The percentage of carbon and hydrogen is calculated as:

Let

The weight of the coal sample taken $= x$ g

Increase in weight of $CaCl_2$ bulb (wt. of H_2O) $= a$ g

Increase in weight of KOH bulb (wt. of CO_2) $= b$ g

Percentage of carbon is calculated:

$$\underset{12}{C} + O_2 \longrightarrow \underset{44}{CO_2}$$

44 g of CO_2 contains carbon $= 12$ g

b g of CO_2 contains carbon $= \frac{12}{44} \times \frac{b}{x} g$

$\therefore$ Percentage of carbon $= \frac{12}{44} \times \frac{b}{x} \times 100$

Percentage of hydrogen is calculated:

$$\underset{2}{H_2} + \frac{1}{2} O_2 \longrightarrow \underset{18}{H_2O}$$

18 g of H_2O contains hydrogen = 2 g

a g of H_2O contains hydrogen $= \frac{2}{18} \times \frac{a}{x} g$

$\therefore$ Percentage of hydrogen $= \frac{2}{18} \times \frac{a}{x} \times 100$

(ii) Percentage of nitrogen: Nitrogen in the sample is estimated by Kjeldahl's method. A known weight of coal sample is heated with concentrated H_2SO_4, K_2SO_4, and $HgSO_4$ for 2-3 hours so that nitrogen is converted into ammonium sulphate.

$$\text{Nitrogen} + H_2SO_4 \xrightarrow{\text{Heat}} (NH_4)_2SO_4$$

The contents are then transferred to a round bottom flask and diluted with water. The solution is then made alkaline with NaOH and liberated ammonia is absorbed in a known volume of standard acid solution. The unreacted acid is determined by back titration with the standard solution of NaOH. The percentage of nitrogen in the coal can be determined by knowing the volume of acid used and ammonia liberated as:

$$\text{Percentage of nitrogen} = \frac{\text{Volume of acid used} \times \text{Normality} \times 1.4}{\text{weight of coal taken}}$$

(iii) Percentage of sulphur: It is determined from the washings obtained from the known mass of coal used in a bomb calorimeter for the determination of calorific value. During this process, sulphur is converted into sulphuric acid. The washings are treated with $BaCl_2$ solution when barium sulphate is precipitated. The precipitate is filtered, washed and heated to constant weight. The percentage of sulphur is calculated as:

$$\text{Percentage of sulphur} = \frac{\text{wt. of } BaSO_4\text{ppt}}{\text{wt. of coal taken in bomb}} \times \frac{32}{233} \times 100$$

(iv) Percentage of oxygen: It is calculated as:

Percentage of oxygen = 100 – (Percentage of all other elements)

5.3.2 Grading of Coal

Grading of coal is different from its classification. Grading is done for fixing its price. Coal containing low moisture is graded on the basis of its ash content, while coal containing high moisture is graded on the basis of its ash and moisture contents. The grading of coal is shown in Table 5.2

Table 5.2 *Coal commissioner's Grading Scheme*

Non-Coking Coal				**Coking Coal**		
Grade	For high moisture or Raniganj coal (Ash + Moisture)	Low moisture or other than Ranigang coal (Ash%)		Grade	Ash content (%)	
Selected A	Under 17.5	Under 15		A	Under 13	
Selected B	17.5 – 19.0	15 – 17		B	13 – 14	
Grade I	19.0 – 25.0	17 – 20	Moisture content is less than 2%	C	14 – 15	These have moisture less than 2%
Grade II	25.0 – 25.0	20 – 24		D	15 – 16	
Grade III A	—	24 – 28		E	16 – 17	
Grade IV B	—	28 – 35		F	17 – 18	
				G	18 – 19	
				H	19 – 20	
				HH	20 – 24	

NOTES

5.3.3 Liquid Fuels: Flash Point, Aniline Point, Octane Number and Carbon Residue

Despite the extensive use of liquid hydrocarbons for fuels, the use of liquid fuel dates back to very ancient times, when vegetable and animal oils were burnt for the production of light and to some extent for heat. These oils are of little importance for these purposes today because they have been largely supplanted by petroleum products.

Petroleum is a dark coloured viscous oil found deep in earth's crust. Chemically, it is a mixture of various types of hydrocarbons along with some oxygen, sulphur and nitrogen containing compounds. The average composition of crude petroleum is given below.

C = 80 - 85%, S = 0.1 - 0.5%, H = 10 - 14% ; N and O = Negligible

Petroleum may be classified into several classes depending upon the nature of the hydrocarbon:

(i) **Paraffinic base type:** It is mainly composed of saturated hydrocarbons from CH_4 to $C_{35}H_{72}$.

(ii) **Asphaltic base type:** This type of petroleum contains mainly cycloparaffins or naphthenes.

(iii) **Mixed base type:** It contains both paraffinic as well as asphaltic hydrocarbons.

• Flash point of liquid fuels

Flash point is defined as the minimum temperature at which the combustible liquid gives off sufficient vapours to ignite momentarily when a flame of standard dimension is brought near the surface of liquid for a prescribed rate in an apparatus of specified dimensions. That is the flash immediately disappears for want of more vapours at low temperature.

At a slightly higher temperature, the heat from the flash becomes sufficient to evaporate more liquid and maintain combustion. This lowest temperature (usually 5°C to 40°C higher than the flash point) at which an oil gives off sufficient vapours which when ignited continues to burn for at least five seconds, is called the fire point of the oil.

Liquids having flash point less than 140°F are known as flammable liquids and those with flash point more than 140°F are called combustible liquids.

Determination of flash point

It can be determined using the following apparatuses:

1. Cleveland's open cup apparatus is used for fuel oils having flash point below 175°F. The oil is heated with its surface exposed to air to determine its flash point.

2. Abel's Closed Cup Apparatus Abel's apparatus consists of a cylindrical brass cup surrounded by double jacketed copper water bath which is enclosed in a copper casing mounted on an iron tripod. The space between water bath and oil cup form an oil cup air bath, by means of which the oil can be heated uniformly.

NOTES

The oil cup is provided with a brass cover having arrangement for a small test flame, a sliding shutter covering three small openings in the lid and openings for a paddle stirrer and a thermometer immersed in the oil.

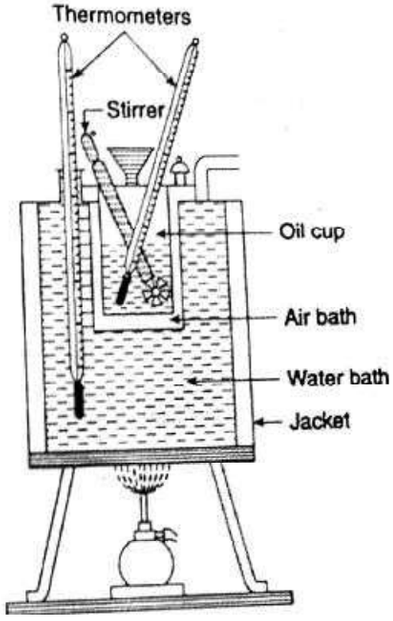


Fig. 5.1 *Abel's Flash Point Apparatus*

Procedure

Fill the oil cup with the oil under test up to the point of gauge. Replace the cover. Fix the oil cup into the apparatus and assemble the paddle stirrer and thermometer with its bulb dipping into the oil. Fill the water bath with cold water. Close the sliding shutter and light of the standard flame. Switch on the heating device. Adjust the rate of heating in such a way that temperature of the oil increases at a rate of 1°C per minute. Stir the oil continuously by turning the paddle stirrer. Stirring should be discontinued only during the introduction of the test flame over the oil surface.

At every degree rise of temperature, open the sliding shutter and introduce the test flame over the oil surface through the central opening to see whether the ascending current of oil vapour gives a flash. Record the minimum temperature at which a distinct flash appears as the flash point of the oil. Abel's apparatus is best suited for oils having flash point below 120°F.

3. Pensky-Marten's closed cup apparatus. Pensky apparatus is most commonly used for the determination of flash points of oils having flash points between 50°C to 370°C. Brass cup which is supported by its flange over a beating vessel. The cover for the cup is provided with four openings of standard dimensions, which are meant for

(i) stirrer,

(ii) standard thermometer,

(iii) an air inlet, and

(iv) a device for introducing the standard flame.

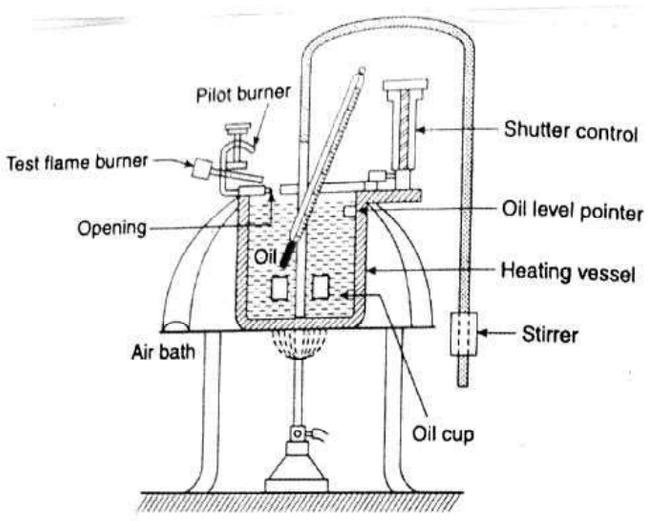


Fig. 5.2 *Pensky Marten's Apparatus*

The shutter provided at the top of the cup has a lever mechanism. When the shutter is turned, openings for the test flame and air are opened and the flame exposure device dips into the opening over the surface of the oil. The test flame gets extinguished when it is introduced into the opening for the test but as soon as it returns to its original position on closing the shutter, the flame is automatically lighted again by the pilot burner.

Procedure

The oil sample under test is poured into the oil cup up to the mark. The cover is fixed on the top. The test flame is lighted and adjusted to the bead size of 4 mm. The apparatus is heated so that the oil temperature increases by 5°C per minute while the stirrer is rotated at about 60 revolutions per minute. When the temperature rises to about 15°C of the anticipated flash point, the test flame is dipped into the oil vapour for 2 seconds at every degree rise of temperature. This is done by twisting the knob which lowers the test flame and opens the shutters The flash point is taken as the minimum temperature at which a distinct flame is observed on introducing the test flame into the oil cup. Oils containing traces of volatile substances are liable to flash below the true flash point of the oil. The minimum flash point required for insulating oils is 145°C and that for turbine oils is 165°C.

Significance of flash point determination

Flash and fire point: ensures safety against fire hazards during storage, transport, handling and use of oil. Flash point of an oil is often used as a means of identification and also for detection of solvent contamination of lubricating oils. It provides a rough guide to the base of an oil since it is higher for the oils of paraffin base than those of naphthenic base.

• Aniline point of liquid fuels

The tendency of a lubricant to mix with aniline is expressed in terms of aniline of a liquid. Aniline point (or standard aniline point) is the lowest temperature at which the oil is completely miscible with an equal volume of freshly distilled aniline.

NOTES

Alternatively, aniline point is the minimum equilibrium solution temperature for equal volumes of aniline and the oil.

Certain lubricants with high aromatic content remain completely miscible with equal volume of aniline and separation into different phases may not be observed even at solidification. To determine such low aniline point, 1. volume of the sample is mixed with 2 volumes of aniline and 1 volume of a diluent like *n*-hexane or n-heptane. Diluent lowers the solubility of aniline with the sample and so with the decrease in temperature, separation of phases can be easily achieved. The equilibrium solution temperature observed under these conditions is called mixed aniline point.

Determination of aniline point of an oil

Equal volumes of aniline and oil are heated to effect complete dissolution and then cooled under controlled conditions. The temperature at which the entire mixture becomes cloudy throughout is called the aniline-point of the lubricating oil.

Procedure

The apparatus is thoroughly cleaned, and dried at 110°C. About 10 mL of pure and dry aniline (dried over pure KOH pellets, filtered and freshly distilled) are taken in a heat resistant pyrex test tube (2.5 cm, 15 cm) and an equal volume of the lubricating oil is added. The tube is fitted with a cork which also holds an electrically operated stirrer and a thermometer in such a way that the bulb of the thermometer is about 5 mm above the bottom of the test tube. The tube is inserted into an outer jacket (4 cm, 17.5 cm). The stirrer is started and the aniline -oil mixture is observed to find whether the miscibility is complete at room temperature itself. If so, the jacket holding the tube is immersed in a non-aqueous cooling bath. Cooling is continued slowly at a rate of 0.5 to 1° C per minute, until the entire aniline-oil mixture suddenly becomes cloudy throughout. This temperature is recorded as the aniline-point of the lubricating oil.

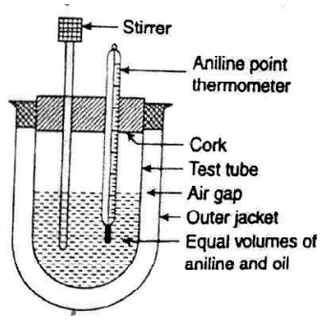


Fig. 5.3 *Aniline Point Apparatus*

If the aniline-lubricating oil mixture is not completely miscible in the tube at the room temperature, then slowly heat the jacket, holding the tube either directly or by immersing in a hot bath. Stirring is continued while raising the temperature, until the aniline-oil mixture is completely miscible. Then the jacket holding the tube is withdrawn from the hot bath.

The stirring is continued, the temperature is allowed to fall at a rate of 1°C per minute. A cold bath may be used for this purpose, if necessary. The temperature, at which the entire aniline oil mixture becomes cloudy or hazy throughout and the outline of the thermometer bulb is obscured, is taken as the aniline-point of the oil sample.

Precautions

Some of the major precautions are as follows:

- Aniline used should be perfectly pure and dry.
- The whole apparatus should be perfectly dry, because even traces of moisture may give errorneously high results.
- Aniline is hygroscopic and hence water should not be used even in cold and hot baths. Non-aqueous, non-volatile and transparent liquids should be used
- Aniline is highly toxic and hence pipettes provided with a ubbe suction bulb or an aspirator should be used.
- Stirring should be done at such a rate that undue splashing of the liquid or formation of air bubbles are avoided.
- If the expected aniline-point is below the dew point of the atmosphere, the space above the aniline-oil mixture in the tube should be filled with dry N.
- The true aniline-point is characterised by a turbidity which increases sharply as the temperature is lowered.

Significance of aniline point determination

Aniline point of a lubricating oil is a measure of its aromatic contents. Hence it gives an indication of the possible deterioration of an oil when it comes into contact with rubber seals, used in the system to prevent leakages. Thus, lower the aniline point of a lubricating oil, the more severe will be its attack on the rubber sealing. Obviously, a higher aniline point is desirable for a lubricating oil, because it means that the oil contains lower percentage of aromatics.

• Carbon residue of liquid fuels

The carbon residue of a lubricating oil is expressed in terms of percentage of carbon that is left on evaporating a known quantity of oil under specified test conditions in a specified apparatus. Carbon deposition in internal combustion engines results both from incomplete combustion of the fuel as well as carbonisation of the lubricating oil. Excessive build-up of carbon deposits in the combustion chamberresults in decreased volume of the charge at the end of the compression stroke giving increased compression ratio which leads to detonation.

Determination of carbon residue

Some of the methods to determine carbon residue are as follows:

1. **Conradson method:** Take two glass beads (2.5 nam) in the silica crucible and weigh. Also weigh 2 g of the oil sample in the crucible and place it in the centre of skidmore crucible. Th dmore crucible is put in the centre of sheet iron crucible containing the levelled layer of sand (fig. 5b.8). It is provided

NOTES

with a lid having a small opening for the escape of vapours. The whole assembly of the crucibles is covered with the hood in order to distribute the heat uniformly. The outer iron crucible is heated strongly till the smoke appears above the chimney. When the vapours cease to burn and no further smoke is observed, the bottom of the crucible is heated to redness. After 10 minutes, the burner is put off and the whole apparatus is allowed to cool until no smoke is seen. The silica crucible is transferred to a desiccator, cooled and weighed. From the weight of carbon deposited in the crucible, the percentage of carbon residue in the oil can be calculated.

Calculation

$$\text{Weight of the crucible + beads} = w_1 g$$
$$\text{Weight of the crucible + beads + oil} = w_2 g$$
$$\text{Weight of the crucible with carbon residue} = w_3 g$$
$$\text{Weight of the oil taken} = (w_2 - w_1)\ \text{g}$$
$$\text{Weight of the carbon residue obtained} = (w_3 - w_1)\ \text{g}$$
$$\text{Percentage of carbon residue in the oil} = \frac{w_3 - w_1}{w_2 - w_1} \times 100$$

2. **Ramsbottom method:** 1 to 4 g of the sample is taken in a stainless steel bulb which is placed in a sheath consisting of an iron tube having a flat closed end. The sheath is immersed in a bath of molten lead which is heated to 550°C for 20 minutes. The bulb is taken out, cooled and weighed. The result is reported as 2% carbon residue to the weight of oil taken for the experiment.

Calculations

$$\text{Weight of the oil taken} = w_1 g$$
$$\text{Weight of the carbon residue obtained} = w_2 g$$
$$\text{Percentage of carbon residue in oil} = \frac{w_2}{w_1} \times 100$$

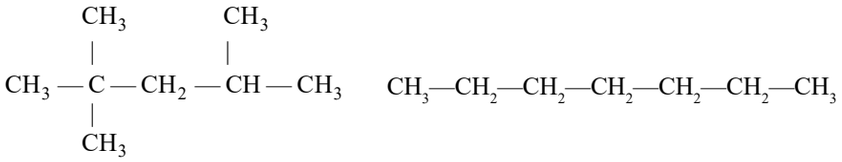
Note: Expected percentage of carbon residue is 2 for the 4 sample taken in the coking bulb.

• Octane number of liquid fuels

The important properties of a fuel for use in automobiles include the boiling range, startibility, vapour pressure and knocking. In an internal combustion engine, the mixture of fuel (gasoline vapour) and air is highly compressed before it is ignited in order to have maximum efficiency'. Ignition of a mixture of gasoline vapour and air by a spark causes a compression wave to pass through the unburnt gas mixture. Under certain conditions this wave can compress the mixture to above its self-ignition temperature, resulting in explosive burning and the characteristic metallic sound called knocking and causing fuel wastage and increased engine wear.

The knocking quality of an automobile fuel is measured in term of the so-called octane number. The hydrocarbon, iso-octane (2, 2, 4–trimethylpentane) which has good resistance to knocking in highest compression motors, is arbitrarily given the octane number of 100, and n-heptane with poor resistance to knocking

is given the arbitrary value of 0 ; the higher the octane number, the better the fuel. All other fuels are compared with their mixtures in suitable proportions.



2, 2, 4–Trimethylpentane (Octane No. = 100)

n-Heptane (Octane No. = 0)

Thus, the octane number of a gasoline is defined as the percentage of iso-octane present in a mixture of iso-octane and n-heptane which matches the fuel (gasoline) in knocking. For example, a motor fuel is said to have an octane number of 70 when it matches in knocking property to a mixture of 30 per cent *n*-heptane and 70 per cent iso-octane. Modern good grade of gasoline has an octane number of 80 which is obtained by blending straight run gasoline with cracked gasoline and hydrocarbons synthesised from refinery gases and adding a small amout of tetraethyl lead.

In addition to tetraethyl-lead (60%), small amounts of ethylene bromide (26%), ehtylene chloride (9%) and a red dye (2%) are also added. About 1-3 ml of this mixture, named as *ethyl fluid*, is added to a gallon of petrol to increase antiknock properties. This type of gasoline is commonly to known as leaded gasoline. The mode of action of TEL in increasing the octane number of gasoline may be any one of the following.

(i) Tetraethyl lead decomposes to metallic lead. The cloud of the so formed fine lead particles acts as a shield between the unburnt charge and the burning portions.

(ii) The finely divided lead particles act as the oxidation centres, thereby making the combustion homogeneous and uniform,

(iii) The metal particles may act as deoxidisers and thus may inhibit the formation of easily detonated peroxides.

(iv) The ethyl free radicals, formed by the dissociation of TEL, convert some of the straight chain hydrocarbons to branched chain hydrocarbons, thus lowering the knocking property of petrol.

The function of ethylene bromide is to remove the deposits of lead, formed by the decomposition of TEL. Ethylene bromide reacts with lead to form lead bromide which is volatile at engine temperature.

$$\underset{\text{thylene bromide}}{Pb + BrCH_2.CH_2Br} \longrightarrow \underset{\text{lead bromide}}{PbBr_2} + \underset{\text{ethylene}}{CH_2 = CH_2}$$

5.3.4 Gaseous Fuels: Water Gas and Produce Gas

These fuels include the following

• **Water Gas:** Water gas is a mixture of carbon monoxide and hydrogen along with a small amount of carbon dioxide. It is obtained by the action of steam on a bed of coke heated to 1000°C.

NOTES

$$C + H_2O \longrightarrow \underset{\text{Wate gas}}{CO_2 + 2H_2} - 28 \text{ kCal}$$

Since the above reaction is endothermic, the coal cools down after a few minutes and the reaction proceeds in a different way to form carbon dioxide and hydrogen instead of water gas ($CO + H_2$).

$$C + 2H_2O \longrightarrow CO_2 + 2H_2 - 19 \text{ kcal}$$

In order to avoid the above undesirable reaction, the current of steam is intermittently replaced by a blast of air. The following reactions now occur.

(a) $C + O_2 \longrightarrow CO_2 + 97$ kcal

(b) $2C + O_2 \longrightarrow 2CO + 59$ kcal

Thus due to exothermic reaction the temperature of carbon again rises and when the temperature increases to 1000°C air entry is stopped and steam is again passed. Thus in modem gas plants steam and air are blown alternatively. The period of steam blow (cold blow) is usually 4 minutes while the period of air blow (hot blow) is very short (about 1-2 minutes). The durations of these periods are adjusted in such a way that the maximum yield of water gas is obtained.

A water gas generator is a cylindrical vessel made of steel. It is about 4-5 m in height and 2-3 m in diameter. At the top it is provided with a hopper for adding coke. Water gas outlet is provided near the top. It has two separate inlets for blowing steam and air at will. At the bottom, it is provided with an arrangement for taking out ash formed.

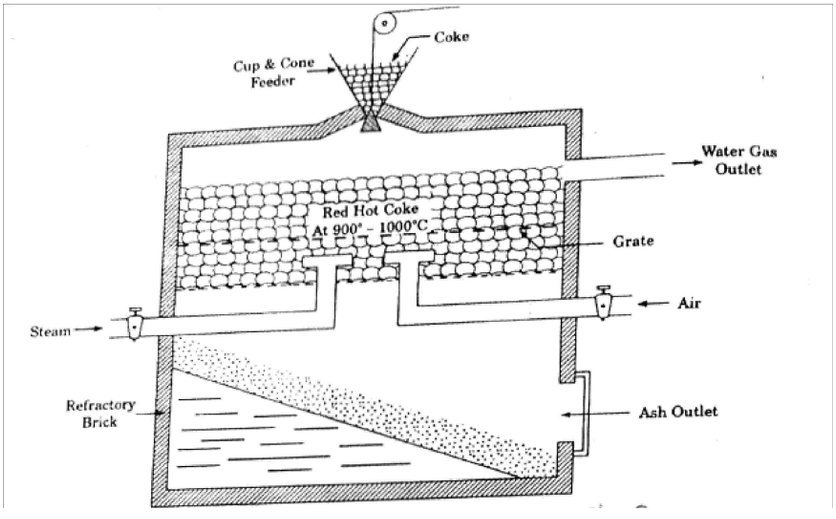


Fig. 5.4 *Manufacture of Water Gas*

During the steam blow, water gas is produced. This is led out through the water gas outlet. On the other hand, nitrogen, carbon dioxide and carbon monoxide are formed during the air blow. These are allowed to escape into the atmosphere. Thus the manufacture of water gas is intermittent.

Properties and composition

Water gas burns with a non-luminous blue flame and hence it is also called blue water gas. It calorific value is about 3255 kcal/m^3. It burns with a high temperature flame about 1200°C. The approximate composition of the water gas is as below

(i) Hydrogen = 48.0%

(ii) Nitrogen = 3%

(iii) Carbon monoxide = 44.0%

(iv) Methane = 0.8%

(v) Carbon dioxide = 4.2%

Uses

Some of the uses are as follows:

(i) Because of its high calorific value, water gas is used as good fuel.

(a) $CO + O_2 \longrightarrow CO_2 + \text{Heat}$

(b) $H_2 + \frac{1}{2} O_2 \longrightarrow H_2O + \text{Heat}$

(ii) It is a good source of hydrogen on commercial scale.

(iii) A mixture of water gas and producer gas is used for the manufacture of ammonia by Haber's process.

(iv) Mixed with hydrogen it is used in the manufacture of methyl alcohol.

$$CO + 2H_2 \xrightarrow[200-300 \text{ atmos. pressure}]{ZnO \text{ (catalyst)}} CH_3OH$$

(v) Water gas is also used for the preparation of carburetted water gas (water gas + gaseous hydrocarbons) which can be used for lighting and heating purposes.

(vi) It is also used for welding purposes.

• **Producer Gas:** Producer gas is a mixture of carbon monoxide and nitrogen. It is produced by burning a solid carbonanceous fuel (coke, etc.) in a limited supply of air. Since low grade coal can be used in producing producer gas, its cost comes out to be very economical. Actually it is considered as the cheapest gaseous fuel. But due to the presence of a large proportion of nitrogen, its calorific value is the lowest (1209 kcal/m^3) of all gaseous fuel.

Manufacture

The furnace used for the manufacture of producer gas is known as producer. It consists of a large air-tight cylindrical vessel of mild steel lined inside with fire bricks. It is about 2–4 m in diameter and 3–5 m in height. At the bottom, it is provided with pipe for blowing air and an arrangement for removing ash. Coal is added through a hopper at the top and producer gas comes out from an exit near the top.

NOTES

When air mixed with a little steam is passed through the inlet, carbon (of the coal) combines with oxygen (of the air) in the lower part of the furnace to form carbon dioxide. Carbon dioxide, so formed rises up through the red hot coal and gets reduced to carbon monoxide during its passage.

$$C + O_2 \longrightarrow CO_2 + 97 \text{ kcal } (\textit{In the lower part})$$

$$CO_2 + C \longrightarrow 2CO - 39 \text{ kcal } (\textit{In the upper part})$$

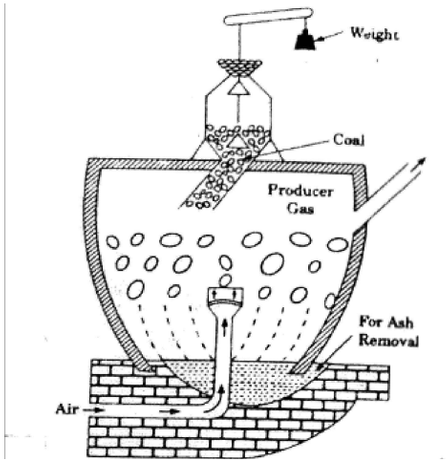


Fig. 5.5 *Manufacture of Producer Gas*

Nitrogen (of air) remains unaffected throughout the process. Thus a mixture of carbon monoxide and nitrogen with traces of carbon dioxide and hydrocarbons comes out through the exit at the upper end of the producer. Since more heat is liberated during the first of the above reactions than is absorbed during the second, in the long run some excess heat is obtained which keeps the coal hot in the product.

Composition and uses

It is a poisonous gas, insoluble in water and heavier than air. The average composition of producer gas is as below:

(i) CO = 22 – 30% ,

(ii) H_2 = 8 – 12%,

(iii) N_2 = 52 – 55%

(iv) CO_2 = 3%,

(iv) Methane = Small amount

As mentioned earlier, it is the cheapest fuel gas and used for the following purposes.

(i) It is used as a fuel for heating open-hearth furnaces (used in steel and glass manufacture), muffle furnaces, retorts (used in coke and coal gas manufacture), etc.

$$2CO + O_2 \longrightarrow 2CO_2 + \text{Heat}$$

(ii) It provides a reducing atmosphere in certain metallurgical operations.

Check Your Progress

5. Define fuel.
6. What is calorific value?
7. Which calorimeter is used for determining the calorific values of gaseous fuels or those liquid fuels which are easily vaporized?
8. What do you understand by flash point?

5.4 CLINICAL CHEMISTRY

Clinical chemistry is the area of chemistry that is generally concerned with analysis of bodily fluids for diagnostic and therapeutic purpose. It is an applied form of biochemistry. There are often variety of different methods and instruments in use for the analysis of a particular constituent. When these different methods and instruments are used for carefully analyzing the same control specimen, as in case of assigned value determinations, the assigned values obtained may be significantly different, and these differences may be of clinical importance. In order to determine the causes for such differences, reference systems or reference points are needed. The reference systems for calibration (calibration materials, standard reference materials) and control (control materials), where the matrices of these different materials must have quite different characteristics, are referred to jointly as reference materials. In order to compare methods, reference methods are required with known, high reliability. Because of the amount of specimen material needed, the time needed for analysis and the facilities required, reference methods are not suitable for routine analysis. Ideally, standard reference materials are developed first, followed by a definitive method, which is then used to evaluate a candidate reference method. This is currently the case for only a small number of the constituents analyzed in the clinical chemistry laboratory. Limitations of knowledge and technique may necessitate certain compromises, with respect to the ideal characteristics of reference materials and reference methods. Possible compromises are discussed, and the various sources of error associated with them are recorded. However, these compromises can also lead to improvement in the reliability and comparability of analytical results from different laboratories. As an example of such improvement, the results are presented for assigned value determinations by highly qualified reference laboratories on control specimen for inter-laboratory surveys. The decision limits are included and the results are compared with those of the survey participants. Thus, various ways are indicated for the use of reference materials and reference methods to improve the reliability and comparability of analytical result suited to the proper procedure of the test.

5.4.1 Composition of Blood

Blood is a red coloured fluid connective tissue, which circulates in our body. It helps in transporting necessary substances such as nutrients and oxygen to the various parts of the body. It has various primary and secondary functions which provide a means of communication between the cells of different parts of the body

and the external environment. Blood is bright red when its haemoglobin is oxygenated. In your body, the blood circulates through two types of blood vessels. The arteries carry oxygenated blood (blood that has received oxygen from the lungs) from the heart to the rest of the body. The blood then passes through the veins back to the heart and lungs where it receives oxygen again for the new circulation.

Blood is a 'life sustaining fluid' as it performs various functions in the body. Without blood, the organs cannot get proper oxygen and nutrients, which they need for their survival. It fights against infections and gets rid of the body's waste products.

Blood accounts for 7 per cent of the human body weight and an average adult has a blood volume of roughly 5 litres. It is composed of blood cells suspended in a liquid called blood plasma. Plasma, which comprises 55 per cent of blood fluid, is mostly water (90 per cent by volume) and 45 per cent of blood cells. The three main types of cells—red blood cells, white blood cells and platelets.

- **Plasma**

Plasma is the transparent fluid part of blood. It is the straw-coloured liquid in which the blood cells are suspended. It is obtained by the centrifugation of 'anti-coagulated blood' (chemical substance added to blood to prevent clotting). When allowed to stand in a glass tube, the cells sediment down leaving a clear supernatant 'plasma.'

The constituents of plasma are water (90–92%) and other organic and inorganic substances like:

- Plasma proteins – Albumin, globulins (antibodies), fibrinogen.
- Clotting factor– These are required for coagulation of blood.
- Fibrinogen – Synthesis in liver essential for blood coagulation.
- Inorganic salts – Sodium chloride, sodium bicarbonate, potassium, magnesium, iron, calcium, copper, iodine, etc..
- Organic waste materials – Urea, uric acid, creatinine waste products of protein metabolism.
- Nutrients – These are various end-products due to digestion of food such as monosaccharide amino acids, fatty acids, glycerol and vitamins.
- Hormones – They are produced by endocrine glands and travel through blood to the target tissue and organs.
- Gases – Blood carries the respiratory gases (O_2 and CO_2) around the body and gives it to the cells where it is needed, for cellular respiration, to make energy.

Table 5.3 *Composition of Blood Plasma*

Component	Percent
Water	92
Proteins	6–8
Salts	0.8
Lipids	0.6
Glucose (blood sugar)	0.1

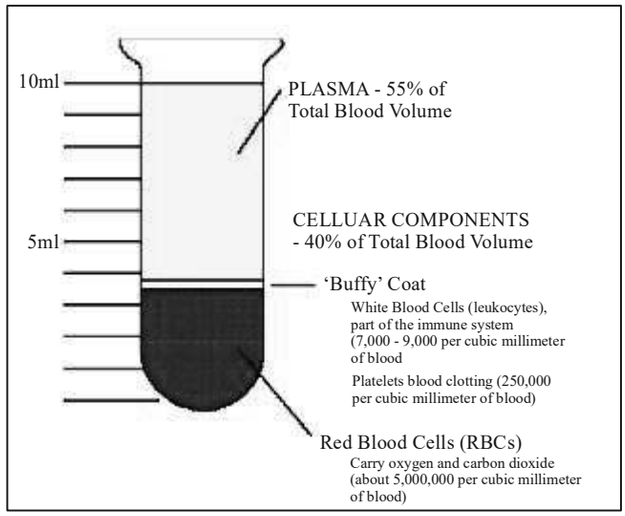
- **Serum**

Serum is the fluid obtained from clotted blood. In blood, the serum is the component that is neither a blood cell nor a clotting factor. It is the blood plasma with the fibrinogens removed. Serum includes all proteins not used in blood clotting and all the electrolytes, antibodies, antigens, hormones and any exogenous substances (e.g. drugs and microorganisms). When blood is allowed to clot in a tube, within 1–2 hours a pale yellow fluid is separated out as the clot is retracted and serum can be taken out. When the blood has clotted firmly and the clot has started to retract, the sample may be left in a refrigerator overnight at 4°C, so that clot retraction may become complete under conditions unfavourable for the growth of bacteria. When serum and cells are required urgently, the sample can be defibrinated. This can be performed by placing the blood in a conical flask with a glass rod on which small pieces of glass capillaries are attached. The blood is rotated inside the flask in a moderate speed and is coagulated within five minutes and most of the fibrin is collected on the glass rod. Hence, when the fibrin formation is complete the difibrinated blood is centrifuged and the serum is obtained. It is done for investigating certain haemolytic anaemia. Serum does not contain clotting factors as they have been used up in the clotting process.

Table 5.4 shows the difference between plasma and serum while Figure 5.8 shows the separation of serum.

Table 5.4 *Difference between Plasma and Serum*

PLASMA	SERUM
(i) It is the fluid part of anti-coagulated blood.	(i) It is the fluid part of clotted blood.
(ii) It contains all the factors like fibrinogen, prothrombin, etc.	(ii) It is devoid of all clotting factors (fibrinogen) as they have been used up in clot formation.
(iii) It is used for coagulation studies like PT and APTT mainly in haematology.	(iii)It is used for the estimation of protein, etc. mainly in biochemistry.



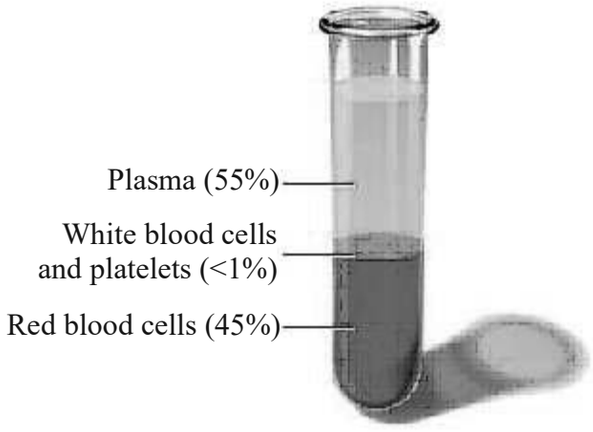


Fig. 5.6 *Separation of Serum and Plasma*

Cellular components of blood

The cells suspended in the plasma are carried to the various parts of the body through circulation. The three types of blood cells are:

- **Erythrocytes (Red blood cells)**

Red blood cells (RBCs were probably first described in the 17th century by a biologist Jan Swammerdam through a microscope. Later, Anton van Leeuwenhoek provided another microscopic description in 1674. This time, a more precise description of red blood cells was provided, even approximating their size, 25,000 times smaller than a fine grain of sand.'

In 1901, Karl Landsteiner published his discovery of the three main blood groups–A, B and C (which he later renamed to O). Landsteiner described the regular patterns in which reactions occurred when serum was mixed with red blood cells, thus identifying compatible and conflicting combinations between these blood groups.

In 1959, by the use of X-ray crystallography, Dr. Max Perutz was able to unravel the structure of haemoglobin, the red blood cell protein that carries oxygen.

The term erythrocytes comes from Greek erythros for 'red' and kytos for 'hollow,' with cyte translated as 'cell' in modern usage.

They are biconcave disc-shaped, non-nucleated cells with a diameter of 7.2 – 7.7 (microns). It is thicker at the periphery than at the centre. The size may decrease or increase in different conditions. The colour of red blood cells is red due to the presence of the red coloured pigment called haemoglobin. Erythrocytes consist mainly of haemoglobin, a complex molecule containing heme groups whose iron atoms temporarily link to oxygen molecules in the lungs and release them throughout the body. Haemoglobin also carries some of the waste product carbon dioxide back from the tissues. Females usually have 4–5 million erythrocytes per cubic millimeter of blood, men have 5–6 million per cubic mm. If the number is considerably less, the person has anaemia and Polycythemia, which is a condition where the haemoglobin is more than the normal range.

It contains haemoglobin. It is a metaloprotein-complex composed of haem and globin. The haem part consists of iron and globin is the protein content. In mammals, the protein makes up about 97 per cent of the red blood cells dry content and around 35 per cent of the total content (including water). Haemoglobin transports oxygen from the lungs to the various parts of body (i.e. the tissues) where it releases the oxygen for cell use. Haemoglobin also transports CO_2 back from the tissues to the lungs where it gets purified.

Haemoglobin has an oxygen binding capacity of 1.36 ml to 1.37 ml O_2 per gram of haemoglobin, which increases the total blood oxygen capacity seventy folds. Haemoglobin is found in the non-erythroid cells of the brain, like the neurons in the substantia nigra, macrophages, alveolar cells and mesangial cells in the kidney. In these tissues, it acts as an antioxidant and a regulator of iron metabolism.

The haem part is made up of a complex protoporphyrin and haem. The synthesis of haem takes place in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. Even after the loss of the nucleus, the residual ribosomal RNA in the reticulocytes allows furthur synthesis of Hb until the reticulocyte enters the peripheral blood circulation. Therefore, only the precursors of erythrocytes can synthesize the haem.

- **Leucocytes (White blood cells)**

Leucocytes are white blood cells, are few in number as compared to the RBCs. They are formed in the bone marrow and the process is known as leucopoiesis. Lymph nodes, thymus and spleen are the other sites of leukocyte synthesis and primarily the lymphocytes are the cells which are formed in these organs.

Leukocytes are spherical in shape and slightly larger than red blood cells. There are five types of leukocytes found in the peripheral blood circulation. They are the neutrophils, eosinophils, basophils known as the 'granulocytes' and the other two, the lymphocytes and monocytes called 'agranulocytes.' Leukocytes

act as a defense against 'foreign' material like infectious agents, foreign bodies and abnormal proteins. In the presence of a foreign material, basophils and some lymphocytes release chemicals that cause inflammation and trap the invaders. The neutrophils act against the foreign body and engulf them. This process of digestion is called 'phagocytosis.' In addition to neutrophils (which are a lot in number), eosinophils and monocytes, also assist in phagocytosis.

Lymphocytes play an important role in the immune mechanism as they produce antibodies against various diseases. Lymphocytes begin to multiply and get transformed into plasma as soon as microbes enter the human body. Each microbe stimulates only one type of lymphocyte to multiply and form one type of plasma cell. The plasma cells makes a specific antibody to destroy the particular microbe that has invaded the body. The reserves of white blood cells are released and the manufacturing of large quantities of the appropriate white cells begins as soon as a parasite or virus enters the body.

The WBCs morphologically and functionally differ from the erythrocytes as:

(i) They have no haemoglobin.
(ii) They are bigger in size.
(iii) They are nucleated and amoeboid.
(iv) There are several varieties with different functions.

The total leukocyte count is defined as the number of leukocytes per micro litre of blood and is of utmost diagnostic importance as it assists in diagnosis of various kinds of diseases/ailments. A healthy person should have a WBC count in the range of 4,000–11,000 cells/cubic mm. A rise in WBC count in blood is called leucocytosis while a fall in WBC count is called leucopenia.

The two main types of WWCs are as follows:

(i) Granulocytes

It includes thye following:

1. **Neutrophil:** It is also know as polymorph nuclear leukocyte. Neutrophil is derived from the Latin word *neuter* meaning neither and the Greek word *philein* meaning to love, thus neutrophil means 'love neither.' This is a reference to the fact that Neutrophils stain easy with neutral dyes.

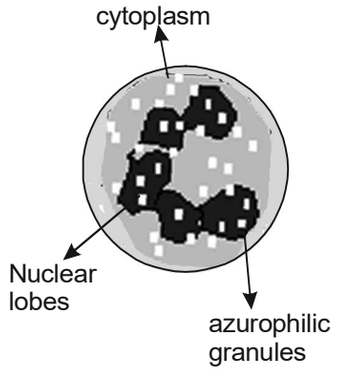


Fig. 5.7 *Neutrophil*

The cells have a diameter of 10–12 microns. When stained with Leishman's stain, it contains granules present in cytoplasm which stain pink. These are fine granules. The nucleus contains segments also called lobes which vary from 2–5 lobes. Neutrophils constitute about 40–75% of the WBCs. A main characteristic of neutrophils is that they have three to five rounds of nuclear lobes within them that are connected by thin threads known as 'chromatin.' Neutrophils stain easily when exposed to neutral dyes and their nucleus will take a dark purple/blue stain. The cytoplasm of a neutrophil will appear light pink with purplish pink granules enclosed in it due to primary or azurophillic granules.

Neutrophils have a phagocytic function, capable of ingesting micro organisms or particles. They can invade and kill any micro organism entering the body. Neutrophils undergo a process called chemotaxis, which allows them to migrate towards sites of infection or inflammation. The granules in the neutrophil contains certain enzymes, which are involved in the killing of ingested microbes and further digesting them. The primary granules which are slightly basophilic in nature are mainly peroxidases and acid hydrolytic enzymes. The secondary granules contain the alkaline phosphatase and certain other enzymes. Peroxidase is a neutrophilic protein which helps in the microbicidal function of the neutrophil. Neutrophil alkaline phosphatase enzymes are present abundantly in mature neutrophil. It can be demonstrated by the cyto-chemical staining of neutrophil and its activity is scored in the blood smear. Neutrophil alkaline phosphatase (NAP) may be raised in conditions like bacterial infection, trauma, myocardial infarction, hormonal contraceptives, etc. NAP is lowered in conditions like chronic myloid leaukaemia, paroxysmal nocturnal haemoglobinuria, infectious mononucleosis, etc.

Cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin-8 (IL-8), interferon gamma (IFN-gamma) and C5a, which these cells use to direct the path of their migration.

2. **Eosinophils:** The word 'Eosinophil' is derived from the Greek word *eos* meaning 'dawn,' and 'philein' meaning 'to love.' Thus, the two words together form 'to love dawn.' Eosinophils become stained when exposed to a dye known as eosin. This is why they are called 'eosinophils.'

   Eosinophils are motile larger than neutrophils (12–17 micron in diameter) and have coarse, deep red to orange staining granules in cytoplasm and two nuclear lobes. The Eosinophils are present as 1–6%. Eosinophils are white blood cells that are the most important components of the immune system responsible for combating multicellular parasites, allergic reactions and certain infections. Along with most cells, they also control mechanisms associated with allergy and asthma. They are involved in reactions to foreign proteins and to antigen – antibody reactions. The Eosinophils increase if you have allergic disorders, parasitic infections etc.

NOTES

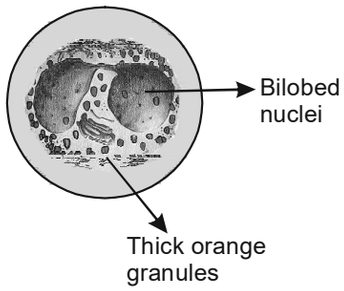


Fig. 5.8 *Eosinophils*

3. **Basophils:** The word 'Basophil' originated from the Greek word *basis* meaning 'foundation' and the Greek word 'philein' meaning 'to love.' The two words together form 'to love foundation.'

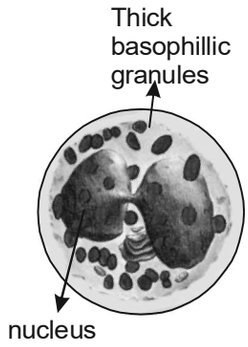


Fig. 5.9 *Basophils*

Basophils are cells that have coarse, basophilic granules present in the cells which often cover the nucleus. Basophils are distinguished by their large purplish black granules that usually cover the nucleus. The granules of basophils contain heparin and histamine which has an affinity for the basic dyes. The basophils are about 0-1% in the blood. Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed. Basophils play a role in both parasitic infections and allergies. They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macro parasite defense and allergy. Basophilia is a condition of increased basophil count in the blood. The causes of Basophilia are chronic myeloid leukaemia, polycythaemia vera, myelofibrosis and certain hyper sensitivity state.

(ii) Agranulocytes

It includes the following:

1. **Monocytes**: There are large, circulating, phagocytic white blood cells, having a single well-defined kidney shaped indented nucleus present in the cells and the cytoplasm is abundant grey blue which may contain fine granules and vacuoles. Monocytes measure 12–20 micron and constitute about 2–10% in blood. Monocytes leave the blood and become macrophages in

the tissue. Macrophages are large, phagocytic cells and their function is to engulf foreign material (antigens) that enter the body and the dead and dying cells of the body. Hence, monocytes have an important function of phagocytosis of foreign material entering the cell. The blood monocyte and tissue macrophage make up a mono nuclear phagocyte system (reticuloendothelial system). This is important in defense against microorganisms. The cells are motile and respond to chemo tactic factors, become immobilized and engage in phagocytosis, a process where the particle is coated with IgG or complement and kill the ingested microorganism.

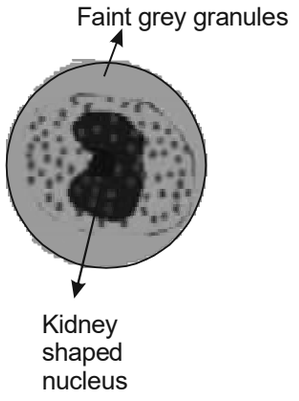


Fig. 5.10 *Monocytes*

Monocytosis, an increase in the number of monocytes in the blood. It often occurs during chronic inflammation and the diseases that produce this state are, as follows:

Infections: It includes the given discusses.

- Bacterial: Tuberculosis, brucellosis, sub-acute bacterial endocarditis, typhoid fever
- Rickettsial: Typhus, rocky mountain fever
- Protozoan – Malaria, Kalaazar, trytanosomiasis
- Viral – Infectious mononucleosis

Neoplasm: It includes the given discusses.

- Hodgkins Lymphoma
- Monocytic Leukaemia
- Myeloproliferative disorders
- Multiple Myeloma

Miscellaneous: It includes the given discusses.

- SLE
- Sarcoidosis
- Haemolytic Anaemia
- Hypochromic Anaemia

NOTES

2. **Lymphocytes:** Lymphocyte comes from the Latin word *lympha* meaning 'spring water' and the Greek word *kytos* meaning 'cell.' Put together, it forms 'spring water cell.'

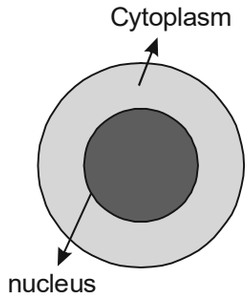


Fig. 5.11 *Lymphocytes*

The lymphocytes are mainly of two types, small lymphocytes and large lymphocytes. A small lymphocyte measures about 9–12microns diameter and a large lymphocyte measure 12-16 microns in diameter. The most common types of lymphocytes are:

- **B lymphocytes (B cells):** These are responsible for making antibodies.
- **T lymphocytes (T cells):** There are several subsets of these such as:
  - o **Inflammatory T cells**: They recruit macrophages and neutrophils to the site of infection or other tissue damage.
  - o **Cytotoxic T lymphocytes (CTLs):** They kill virus-infected tumour cells.
  - o **Helper T cells**: They enhance the production of antibodies by B cells.

The morphology of lymphocyte is round cells which include nucleus and coarse clematis and scanty basophilic cytoplasm. Mostly, the circulating lymphocyte in PBS is small but large may also be present. The Lymphocytes are 20–45% in blood. Their main function is production of antibodies which provide immunity to the body. The lymphocytes are also clarified as T and B lymphocytes depending on where they originate from.

If they are derived from the bone marrow, they are called as B-lymphocyte and if they derived from thymus, they are called T–lymphocyte. The function of T and B lymphocyte is to provide cell mediated and humoral immunity.

- **Thrombocytes (Platelets)**

Platelets, also known as thrombocytes are small, irregularly-shaped nuclear cells 2–3 micron in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is between 8 to 10 days. The main function of platelets is hemostasis, which is maintenance of blood flow. When a blood vessel breaks, platelets gather in the area and help seal off the leak and help in clotting of blood. Certain proteins called clotting factors are critical to the clotting process. Although platelets alone can plug small blood vessel leaks and temporarily stop or slow bleeding, the action of clotting factors is needed to produce a strong, stable clot.

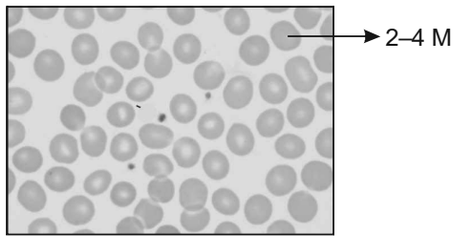


Fig. 5.12 *Platelets*

The process of clotting is like a puzzle with interlocking parts. When the last part is in place, the clot happens — but if even one piece is missing, the final pieces cannot come together. The life span is up to 10 days in circulation and the count is about 1.5lac- 4.5lac/cubic mm in peripheral blood. When the count is reduced, it is called thrombocytopenia. They are involved in haemostasis of blood

The chief functions of platelets are:

(i) Platelet adhesion
(ii) Platelet aggregation
(iii) Platelet release reaction
(iv) Clot retraction

5.4.2 Collection and Preservation of Samples

To perform various tests in a laboratory, we require blood. Blood is collected in various forms:

- Venous puncture
- Skin puncture
- Arterial puncture

In haematology, the blood vessels are the main source of blood. Venous blood is obtained by vein puncture, capillary blood is obtained by skin puncture and arterial blood is obtained by arterial puncture. Out of these three, venous blood is preferred as it is easy to collect the sample from the patient and a number of investigations can be done from the drawn blood sample. The various points which should be kept in mind during collection of blood are as follows:

- There should be an appropriate patient identification method, equipment selection, procedure and usage procedure.
- There should be a proper labelling procedure and completion of laboratory requisitions.
- Sample drawing sequence should be always kept in mind.
- Preferred venous access sites should be used, and all the factors should be considered in site selection, and you should have the ability to differentiate between the feel of a vein, a tendon and an artery.
- Patients should be cared for after the completion of the vein puncture procedure.
- Safety and infection control procedures should be implemented thoroughly.
- Quality assurance issues should be taken into consideration.

NOTES

Sample labelling

A properly labelled sample is very essential so that the results of the test of any patient can be easily distinguished. The key elements in the labelling of any sample are:

- A patient's surname, first and middle name
- Patient's ID number
- Date, time and initials of the phlebotomist must also be on the label of each sample.

Procedure of sample collection

Following points must be kept in mind while collecting a sample:

- The phlebotomist should be a courteous, and understanding person and behave nicely with all patients. He should greet the patient and identify himself and explain the procedure which will take place.
- Effective communication is needed, both verbally and nonverbally because the patient who is at ease and is comfortable will concentrate less on the procedure and will be more cooperative.
- Always thank the patient and excuse yourself courteously when the procedure is finished.
- The patient should be properly positioned so that he can either sit in a chair, lie down or sit up in the bed.
- The patient's arm should be hyper extended and he should make a fist after selecting the vein for phlebotomy.
- Apply the tourniquet (a rubber tube to build the pressure on the vein to make it prominent) just before drawing the blood sample. The tourniquet should be applied 3–4 inches above the selected puncture site. Do not place it too tightly or leave it for more than 2 minutes.
- The skin should be cleaned with 70% alcohol and allow it to dry before the puncture.
- If punctured wet it will cause irritation as the alcohol might seep inside the vein.
- Puncture the site with needle and syringe at the angle of 15–30 degree with the surface of arm.
- Slowly insert the needle through the skin into the lumen of the vein.
- Release the tourniquet before taking out the needle from the patient's arm.
- Remove the needle from the patient's arm by using a swift backward motion.
- Apply cotton on the puncture site once the needle is out of the arm and apply adequate pressure to avoid formation of a hematoma.
- Remove the needle from the syringe and fill the containers with the blood sample.
- Mix the sample gently with the anticoagulants already present in the containers.

- Dispose the used needle in the incinerator and dispose other contaminated materials in designated containers.
- Label all the appropriate tubes immediately.
- Deliver the specimen promptly to the laboratory.

Additional Considerations

Some of the considerations ons are as follows:

1. To prevent a hematoma

The point must be kept in mind are as follows

- Only the uppermost wall of the vein should be punctured.
- The tourniquet should be removed before the needle is removed.
- Only the major superficial veins should be used.
- It should be made sure that the needle completely penetrates the upper most wall of the vein (Partial penetration might allow the blood to leak into the soft tissue surrounding the vein by the needle bevel).
- Pressure should be applied to the vein puncture site to prevent hemolysis (which can interfere with many tests)
- Mix tubes containing anticoagulant additives slowly 5-10 times. Hemolysis is the release of the haemoglobin after the red blood cells have been ruptured .The Plasma or serum will appear pink to red if the sample undergoes Hemolysis. It is necessary to avoid Hemolysis during the sample collection, transportation and storage.
- Drawing blood from a hematoma should be avoided.
- Avoid drawing the plunger back forcefully, if using a needle and syringe and avoid frothing the sample.
- Make sure that the vein puncture site is dry.

2. In case of indwelling lines or catheters insertion

The point must be kept in mind are as follows

- Most lines are flushed with a solution of heparin to reduce the risk of thrombosis.
- Discard a sample at least three times the volume of the line before a specimen is obtained for analysis.

3. Hemoconcentration:

The point must be kept in mind are as follows

An increased concentration of larger molecules and formed elements in the blood may be due to several factors:

- Prolonged tourniquet application (not more than 2 minutes)
- Massaging, squeezing or probing any site
- Long-term IV therapy.

NOTES

4. Prolonged tourniquet application

The point must be kept in mind are as follows

- It will lead to hemoconcentration. The hydrostatic pressure causes some water and filterable elements to leave the extracellular space.
- Significant increase can be found in total protein, total lipids, cholesterol and iron.
- It will affect packed cell volume and other cellular elements.

Storage of blood specimens and its effects

The points that must be considered while preserving of blood specimens are as follows:

- When blood is allowed to stand in vitro at room temperature certain changes take place.
- The red cells starts to swell; the mean cell volume (MCV) increases, also osmotic fragility and prothrombin time increases gradually. The erythrocyte sedimentation rate decreases. The leukocytes and platelet counts gradually fall with time.
- When blood is refrigerated at 4°C changes takes place more slowly so that blood may safely be allowed to stand overnight in the refrigerator.
- It is advisable to count platelets and leukocytes within 2 hours of withdrawing the sample and it is best to make a blood film as soon as possible after collection. The fall of leukocyte count within two hours of collection becomes more significant if there is an excess amount of EDTA added to the blood sample. In the automated differential count of WBC, degenerative changes in the leukocyte become prominent and long standing.
- Haemoglobin count can be done even after 24 hours unless the blood does not get turbid or discoloured.
- Blood after being taken out from refrigerator should be allowed to stand on room temperature before performing the test it is mixed gently by rotation for at least 2 minutes. But a refrigerated serum specimen can be used for biochemical analysis.
- For bacterial culture, blood should not be refrigerated.
- It is ideal to perform the haematology test on fresh specimen.
- However, it is not always possible so certain preservatives should be added to the specimen so that the results come accurate.
- A blood film fixed in methanol can be kept for long time, but irrespective of the anticoagulants added blood films start to show changes after 3 hours and by 12- 18 hours it becomes striking. The red cells start showing crenation and sphering, if kept for more than 6 hour at room temperature.
- Few neutrophils show changes in nuclei when they become darkly stained
- Nuclear lobes become separated and few vacuoles develop within the cytoplasm. The lymphocytes too undergo changes to show vacuoles in cytoplasm and nuclear budding.

A specimen, when stored at room temperature in EDTA, causes the red cells to swell and the various laboratory parameters show changes. So, it is advisable to perform the test as soon as possible.

Check Your Progress

9. What is blood?
10. What are the functions of blood?
11. Define plasma.
12. Define serum.
13. Name the cellular components of blood.

5.5 CLINICAL ANALYSIS

Clinical analysis refers to all the tests conducted in a clinical laboratory for making a medical diagnosis. The purpose of this analysis is to identify the measurements of materials and substances. Examination of biological materials like urine, blood, or cerebrospinal fluid is an example of clinical analysis. Clinical analysis is performed on a variety of samples like whole blood (e.g. in hematology), serum, from clotted blood, or plasma from anticoagulated blood, urine, other fluids, feces, tissues, and occasionally on other materials. Normally analytes are measured in serum, as it is easily accessible and is used to reflect target concentrations in tissue or receptors. Generally urine is examined to assess renal tract function and integrity.

All biochemical tests come under chemical pathology. Tests can be performed on any kind of body fluid, but mostly on serum or plasma. Serum is the yellow watery part of blood that is left after blood has been allowed to clot and all blood cells have been removed. Normally it is easily done by the process of centrifugation, which packs the denser blood cells and platelets to the bottom of the centrifuge tube, leaving the liquid serum fraction resting above the packed cells. This initial step before analysis has recently been included in instruments that operate on the "integrated system" principle. Plasma is in essence the same as serum, but is obtained by centrifuging the blood without clotting. Plasma is obtained by centrifugation before clotting occurs. The type of test required dictates what type of sample is used.

5.5.1 Serum Electrolytes

Electrolytes are minerals which are present in the blood and body tissues. They are essential for metabolism, for proper nerve and muscle functioning, for maintenance of proper water balance, and proper blood pH (acid-base balance). The serum electrolyte test includes a group of tests to measure the electrolytes such as Sodium (Na+), Potassium (K+) and Chloride (Cl-). An electrolyte panel, also known as a serum electrolyte test, is a blood test that measures levels of the body's main electrolytes. The serum electrolyte test is performed as a part of a routine health checkup and it help in assessing the problems in the water and pH balance of the body. Electrolytes play an important role in a number of body functions like metabolism, neuromuscular functioning, maintaining hydration and

NOTES

pH (acid-base balance).The serum electrolyte test measures the following electrolytes:

Sodium (Na+): Sodium is one of the essential body electrolyte, along with potassium, chloride, bicarbonate, etc., it helps to maintain the pH balance and normal fluid of the body. It is also vital for cellular metabolism, and in the activity of nerves and muscles and transmission of impulses between them. Sodium is present in all the body fluids. The highest concentration of sodium is found in blood and extracellular fluid. Sodium requirements of the body are principally fullfilled through dietary salt (sodium chloride or NaCl), and a small portion of sodium is absorbed through other food items. The required amount is absorbed and utilized by the body and the remaining is excreted by the kidneys through urine as waste.

Potassium (K+): Potassium is also an important body electrolytes along with sodium, chloride, bicarbonate, etc. It helps to regulate the amount of fluids present in the body and to maintain a correct pH balance. Potassium performs an important role in cellular metabolism and helps in transportation of nutrients and waste products between the cells. It also plays important role in the transmission of nerve impulses to muscles and muscle activity. The amount of potassium required by the body is usully absorbed from dietary sources, and the remaining unabsorbed potassium is excreted by the kidneys. The aldosterone hormone maintains the body potassium level within a small normal range. Aldosterone acts on the nephrons present in the kidneys and activates a sodium-potassium pump which helps the body to reabsorb sodium and excrete potassium. This helps to maintain the potassium concentration in the blood within its normal range. Deviation of potassium concentration from its normal range gives rise to Hyperkalemia (high potassium level in blood), or Hypokalemia (low potassium level in blood). Both these conditions may produce a number of symptoms, and may even be fatal if not controlled.

Chloride (Cl-): Chloride is a requisite mineral which acts as an electrolyte along with potassium, sodium, bicarbonate, etc. It helps to sustain the normal fluid and electrolyte balance in the body. It also function as a buffer to help keep the pH balance of the body. It also plays crucial roles in metabolism. Chloride is used by the stomach to produce hydrochloric acid (HCl) for digestion. Chloride is present in every body fluid. The highest concentration of chloride is found in blood and extracellular fluid (fluid present outside the cells).Most of the chloride is supplied to the body through dietary salt (sodium chloride or NaCl), and a small portion is absorbed through other food items. The required portion is absorbed by the body and the remaining is excreted by the kidneys through urine. The concentration of chloride in blood is maintained within a very narrow range by the body. Its increase or decrease is directly correlated with the sodium levels.

5.5.2 Blood Glucose

Carbohydrates or saccharine are essential components of all living organisms and are, in fact, the most abundant class of all biological molecules. The name carbohydrates, which literally means 'carbon hydrate', stems from their chemical composition which is roughly $(CH_2O)_n$. These also are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols or compounds which yield these derivatives on hydrolysis.

NOTES

Utilization of glucose

Glucose is utilized within the body for different purposes, some of which are as follows:

- **Storage:** In the absence of urgent demand of glucose i.e., energy in the body, the excess glucose may be deposited as glycogen in the liver via a process called glycogenesis. Since the amount of glucose stored in the liver is limited, excess glucose is also converted to fatty acids and stored as triglycerides in the fat depots.
- **Oxidation:** Glucose is completely oxidized in all tissues to produce CO_2 and H_2O for physiological demands of energy. However, under special circumstances in muscle (anaerobic conditions) there is only partial degradation of glucose forming lactic acid.
- **Conversion to fat:** Glucose is converted to fatty acids when glycogen storage is exceeded. The conversion of glucose to fatty acid is irreversible but transformation of glucose to glycerol is reversible.
- **Conversion to amino acids:** Glucose also produces certain amino acids during metabolic processes. These amino acids are said to be glycogenic amino acids i.e. derived from glucose.

In the resting state, the true glucose present in the blood is 60–100 mg/100 ml but this value is higher, i.e., 80–120 mg/100 ml in non–resting state. The condition in which the blood sugar is raised above the normal range is called hyperglycemia and when it goes below the normal level it is called hypoglycemia. The concentration of glucose in the blood depends on two general factors:

- The rate of its entrance into the blood
- The rate of its removal from the blood stream

Sources of blood glucose

Mainly the dietary food is responsible for high blood glucose level. Most carbohydrates in the diet after digestion form glucose, galactose or fructose, which are absorbed in the portal vein. Different glucogenic compounds are also responsible for increasing the blood glucose concentration in the body. For example, lactic acid formed in the skeletal muscle is transported by blood to the liver and kidney where glucose is reformed under the process called as Cori cycle.

In the adipose tissues, the synthesis of triacyl glycerol takes place from glycerol, which is derived initially from blood glucose. Acylglycerols of adipose tissue continually undergo hydrolysis to form free glycerol, which diffuses out of the tissue into the blood. This is converted back to glucose in the liver and kidney by gluconeogenesis. Hence a continuous cycle exists in which glucose is transported to adipose tissue from the liver and kidney and glycerol is returned to be synthesized into glucose by the liver and kidney. Glucose is also formed in the liver from glycogen by process of glycogenolysis. These all factors are mainly responsible for increasing the blood glucose concentration in the body. But the stable glucose level is maintained by the following:

(i) Liver

Liver is the chief organ in the body that maintains the normal glucose concentration. The glucose 6 phosphatases converts glucose 6 phosphate to glucose, which diffuses into the blood stream to form the constant and the only source of blood glucose unless and until glucose is available from the intestine from carbohydrate diet. The lactic acid of the muscle is transported via blood to liver and gets converted to glucose via Cori cycle. The liver cells, unlike other cells, require the oxidation of organic substances to maintain their own vital functioning. In the absence of fuel i.e. glucose, glycogen is diminished, the oxidation of fat occurs which in turn forms keto acids. Some of the keto acids are utilized for cellular energy. During glycogen crisis, the liver cells also utilize arnino acids and protein to meet the energy demand of body via gluconeogenesis.

(ii) Kidney

Kidneys can form glucose by a number of carbohydrate intermediates as well as via gluconeogenesis. But its capacity to produce is less as compared to that of liver. When the blood glucose level exceeds the renal threshold level i.e. 160–180 mg/100 ml, the renal tubules are incapable of reabsorbing all the filtered sugar in glomeruli and the excess glucose is excreted in urine. This results in the decrease of blood glucose concentration.

(iii) Muscles

The extrahepatic tissues are relatively impermeable to glucose, therefore insulin is required for the uptake of glucose to these cells. Increased blood glucose promotes glycogenesis and oxidation of glucose in muscles. Muscle glycogen does not serve directly as a source of glucose during hypoglycemia. But glucose is supplied to the blood from muscle glycogen by Cori cycle or lactic acid cycle. However, muscular exercise promotes the entry of glucose into muscle cells and the glucose is utilized by the muscles. Thus it lowers the blood glucose level.

(iv) Hormones

Different hormones play vital role in the maintenance of blood sugar level. They directly or indirectly help in carbohydrate metabolism and thus affect the glucose level in the body.

5.5.3 Blood Urea Nitrogen (BUN)

Blood urea is a low-molecular-weight waste product derived from dietary protein catabolism and tissue protein turnover, and its levels are inversely correlated with decline in the condition of kidneys. It is a waste product that's created in the liver when the body breaks down proteins. Normally, the kidneys filter out this waste, and urinating removes it from the body. Urea is filtered freely, and a variable amount (H"30%–70%) is reabsorbed predominantly in the proximal tubule, with recycling between the tubule and interstitium in the kidney medulla.

Blood Urea Nitrogen (BUN) is a medical test that measures the amount of urea nitrogen found in blood. The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain 6 to

20 mg/dL (2.1 to 7.1 mmol/L) of urea nitrogen. The test is used to detect renal problems.BUN is an indication of renal (kidney) health. The main causes of an increase in BUN are: high protein diet, decrease in glomerular filtration rate (GFR) (suggestive of kidney failure), decrease in blood volume (hypovolemia), congestive heart failure, gastrointestinal hemorrhage, fever, rapid cell destruction from infections, athletic activity, excessive muscle breakdown, and increased catabolism. Hypothyroidism can cause both decreased GFR and hypovolemia, but BUN-to-creatinine ratio has been found to be lowered in hypothyroidism and raised in hyperthyroidism. The main causes of a decrease in BUN are severe liver disease, anabolic state, and syndrome of inappropriate antidiuretic hormone.

5.5.4 Uric Acid

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$. Uric acid is a normal body waste product. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal component of urine. Uric acid is a chemical created when the body breaks down substances called purines. Purines are normally produced in the body and are also found in some foods and drinks. Foods with high content of purines include liver, anchovies, mackerel, dried beans and peas, and beer. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions, including diabetes and the formation of ammonium acid urate kidney stones. When purines are broken down to uric acid in the blood, the body gets rid of it when you urinate or have a bowel movement. But if your body makes too much uric acid, or if your kidneys aren't working well, uric acid can build up in the blood. Uric acid levels can also increase when you eat too many high-purine foods or take medicines like diuretics, aspirin, and niacin. Then crystals of uric acid can form and collect in the joints. This causes painful inflammation. This condition is called gout. It can also lead to kidney stones.

In humans and higher primates, uric acid (actually hydrogen urate ion) is the final oxidation (breakdown) product of purine metabolism and is excreted in urine, whereas in most other mammals, the enzyme uricase further oxidizes uric acid to allantoin. In humans the normal concentration range of uric acid (or hydrogen urate ion) in blood is 25 to 80 mg/L for men and 15 to 60 mg/L for women.An individual can have serum values as high as 96 mg/L and not have gout. In humans, about 70% of daily uric acid disposal occurs via the kidneys, and in 5–25% of humans, impaired renal (kidney) excretion leads to hyperuricemia. Hyperuricaemia or hyperuricemia is an abnormally high level of uric acid in the blood. Normal excretion of uric acid in the urine is 250 to 750 mg per day (concentration of 250 to 750 mg/L if one litre of urine is produced per day – higher than the solubility of uric acid because it is in the form of dissolved acid urates)

NOTES

5.5.5 Serum Proteins: Albumin and Globulins

Hundreds of proteins are dissolved in the plasma. We can obtain information regarding disease states in different organ systems by measuring the concentration of these proteins. It is done on serum, which is the fluid that remains after plasma has clotted, thus removing fibrinogen and most of the clotting factors. Total protein content provides information regarding the general health of a person. The normal range of serum protein level is 6 to 8 g/dl. Albumin makes up 3.5 to 5.0 g/dl, and the remainder is the total globulins. More than half of the total protein present in serum is albumin. Approximately 30 to 40% of the body's total albumin pool is found in the intravascular compartment. The remainder is extravascular and is located in the interstitial spaces, mainly of the muscles and skin. Albumin is also found in small amounts in different body tissue fluids such as sweat, tears, gastric juice, and bile.

Albumin is a family of globular proteins, the most common of which are the serum albumins. All the proteins of the albumin family are water-soluble, moderately soluble in concentrated salt solutions, and experience heat denaturation. Albumins are commonly found in blood plasma and differ from other blood proteins in that they are not glycosylated. Substances containing albumins are called albuminoids. Albumin does not diffuse freely through intact vascular endothelium. Hence, it is the major protein providing the critical colloid osmotic or oncotic pressure that regulates passage of water and diffusible solutes through the capillaries. Albumin serves in the transport of bilirubin, hormones, metals, vitamins, and drugs. It has an important role in fat metabolism by binding fatty acids and keeping them in a soluble form in the plasma. The binding of hormones by albumin regulates the amount of free hormone available at any time. Because of its negative charge, albumin is also able to furnish some of the anions needed to balance the cations of the plasma.

Albumin is synthesized in the liver. The rate of synthesis is constant in normal individuals at 150 to 250 mg/kg/day, resulting in the production of 10 to 18 g of albumin daily in a 70-kg man. The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease states. Fasting or a protein-deficient diet cause a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual, the liver increases albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein-containing meal.

The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. The globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. Globulins, albumins, and fibrinogen are the major blood proteins. The normal concentration of globulins in human blood is about 2.6-3.5 g/dL. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Globulins

are divided into four groups by electrophoresis. The four fractions are á1, á2, â and ã, depending on their migratory pattern between the anode and the cathode. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney.

5.5.6 Barbiturates

A barbiturate is a drug that acts as a central nervous system depressant. Barbiturates are effective as anxiolytics, hypnotics, and anticonvulsants, but have physical and psychological addiction potential as well as overdose potential among other possible adverse effects. They have largely been replaced by benzodiazepines and non-benzodiazepines ("Z-drugs") in routine medical practice. It is particularly used in the treatment of anxiety and insomnia. The reason for using barbiturates is the significantly lower risk of addiction and overdose. Barbiturates are used for various purposes such as, general anesthesia, epilepsy, treatment of acute migraines or cluster headaches, acute tension headaches, euthanasia, capital punishment, and assisted suicide, etc.

All the barbiturate are chemical derivatives of barbituric acid and that is the reason they are called barbiturate. Barbituric acid itself does not have any direct effect on the central nervous system and chemists have derived over 2,500 compounds from it that possess pharmacologically active qualities. The broad class of barbiturates is further broken down and classified according to speed of onset and duration of action. Barbiturates are classified based on the duration of action. Examples of each class include:

- **Ultra-short acting (30 minutes):** thiopentone, methohexitone
- **Short acting (2 hours):** hexobarbitone, cyclobarbitone, pentobarbitone, secobarbitone
- **Intermediate acting (3-6 hours):** amobarbitone, butabarbitone
- **Long acting (6 hours):** phenobarbitone

Ultrashort-acting barbiturates are commonly used for anesthesia because their extremely short duration of action allows for greater control. The middle two classes of barbiturates are often combined under the title "short/intermediate-acting." These barbiturates are also employed for anesthetic purposes, and are also sometimes prescribed for anxiety or insomnia. The final class of barbiturates are known as long-acting barbiturates (the most notable one being phenobarbital, which has a half-life of roughly 92 hours). This class of barbiturates is used almost exclusively as anticonvulsants, although on rare occasions they are prescribed for daytime sedation. Barbiturates in this class are not used for insomnia, because, owing to their extremely long half-life, patients would awake with a residual "hang-over" effect and feel groggy. Barbiturates can in most cases be used either as the free acid or as salts of sodium, calcium, potassium, magnesium, lithium, etc. Codeine- and Dionine-based salts of barbituric acid have been developed.

NOTES

5.5.7 Acid and Alkaline Phosphatases

Many physiological processes including activity of many lysosomal hydrolytic enzymes are inhibited by heavy metals even though these metals may also activate certain enzymes. Two important phosphatases are Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP), both differing in their sub cellular distribution. ALP activity was found to be highly concentrated in plasma membrane enriched fraction, where as ACP is associated with lysosomes. They have a very significant role in bivalve immunity. These enzymes are involved in a variety of metabolic activities, such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, gonadal maturation, and steroidogenesis. A useful biochemical indicator of lysosomal stability is the specific activity of suitable lysosomal enzyme, and acid phosphatase is a major marker enzyme. Material to be hydrolyzed is taken into lysosomes by endocytosis and the enzymes catalyze the hydrolysis of most of the major polymeric compounds as well as foreign bodies entered into animal body. Lysosomal enzymes are mainly acid hydrolases and ACP is known to hydrolyse the phosphomonoesters, which are produced by hydrolysis of other major phosphates of the cell. ALP is a polyfunctional enzyme, present in the plasma membrane of all cells. It hydrolyses a broad class of phosphomonoester substrates, and acts as a transphosphorylase at alkaline pH_9. It also acts as an early marker of cell differentiation in the osteogenic lineage in bivalve mollusk.

Principle

Alkaline phosphatase splits 4-nitrophenylphosphate into 4-nitrophenol and phosphate in N-methyl 1-D-glucamine buffer. The enzyme activity is measured by the amount of the liberated 4-nitrophenol which can be determined by the constant time method using inhibitor blocking the enzyme active center.

Rate of reaction catalyzed by phosphatase

In the first part of the experiment you will compare the rate of the reaction described above in the presence and absence of phosphatase. Therefore, you will be measuring the rates of both the catalyzed and uncatalyzed reaction which will allow you to determine the degree to which phosphatase increases the reaction rate. In order to express the rate of reaction in units of moles of product produced per unit time you will be comparing the quantity of p-nitrophenolate to the amount of time that elapsed between enzyme addition and NaOH addition. To assay p-nitrophenolate you will use spectrophotometry since this solution absorbs light. The absorption spectrum has a peak at 405nm. Therefore, the first step will be to determine the relationship between Absorbance of light at 405 nm and the concentration of p-nitrophenolate. Following the determination of E you will calculate reaction rate.

NOTES

Influence of pH on phosphatase activity

For most enzymes, pH can influences the catalytic site directly by altering the charge of the protein in this region. The pH will also affect tertiary structure of the enzyme, and may also affect the ability of the enzyme to bind (embrace) the substrate. As a consequence, the catalytic activity, i.e., the degree to which it increases the rate of the reaction) of all known enzymes is sensitive to pH, and each known enzyme exhibits its greatest activity at one pH—the enzyme's optimal pH. In this experiment you will use two different phosphatases, wheat phosphatase and calf phosphatase. These two differ in their pH optima as they are used in very different organs. The calf phosphatase was purified from the intestinal lining; it is normally secreted into the intestines. The wheat phosphatase was purified from seeds; it is stored in lysosomes while the seed is dormant. From your knowledge suggest which enzyme is the acid phosphatase (optimum activity in an acidic environment) and which enzyme is the alkaline phosphatase. In this part, some students will work with the wheat enzyme, some will work with the calf enzyme, and some will receive both enzymes in one solution. Each of you will identify the enzyme(s) you received by determining the effect of pH on enzymatic activity.

Procedure

The maintenance of the target animal and extraction procedure of tissue is same as explained in details. Alkaline and Acid phosphatase activities can be determined. For ALP analysis, reaction mixture containing 0.1 ml of haemolymph and 1ml of PNP (1%) substrate in 0.1 M Glycine/ NaOH buffer at pH-9 will be incubated at 37ºC for 30 min. Reaction mixture for ACP analysis containing 0.1ml of haemolymph and 1ml of PNP (1%) substrate in citrate buffer at pH-4, will be incubated at 37ºC for 30 min. To both the mixtures for ALP and ACP measurements, 1.5 ml of 0.1N sodium hydroxide should be added to stop the reaction. The hydrolytic product, yellow p-nitrophenol, shall be measured at 405 nm in a UV- Vis spectrophotometer. Protein estimation will be performed as per the method of Lowrys *et al*. (1951). Enzyme activity is generally expressed as mg PNP released/ml.

Statistical analysis

Two way ANOVA, followed by comparison test will be used to assess the significant differences between variables. Data are reported as mean and its standard error. The level of significance will be considered at probability level ($P < 0.05$).

Result

Generally, ALP and ACP values revealed an increase in activity with time and concentration on exposure to the heavy metals Copper and Mercury. Alkaline phosphatase activity in copper- exposed animals will show a decrease in all concentrations applied on observation. There might be a drastic increase in activity when compared to healthy control mussels. Highest activity is generally observed in mussels exposed to the highest concentration. A decreased ACP activity has

been detected in copper- exposed mussels on the first day of observation. As the concentration of copper and days of exposure increased, ACP activity also increased.

NOTES

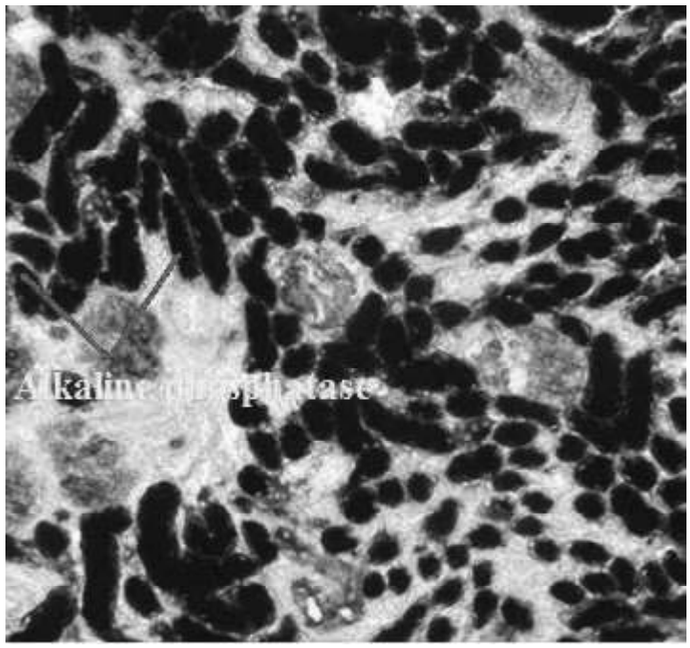


Fig. 5.13 *Alkaline Phosphatase Distribution in Bivalves show Brush Border Appearance*

ACP activity gave the highest value in mussels exposed to 30 µg/L. Low activity of ACP has been reported in *P. viridis* on the 1st day of exposure to mercury. ACP activity was found to be increasing on the 7th day of exposure when compared to the value of the 1st day, though the value remained still lower to that of control mussels. It can be seen that for ALP activity, concentration has no significant impact on the dependent variable at probability level.

Discussion

The increase in activity of ACP and ALP reveals that the animals are under metal stress. Bivalves subjected to metals avoid the toxicant intake by valve closure and hence reducing the filtering activity. The decrease in ACP and ALP activities at the initial stages of exposure to copper and mercury can be attributed to this avoidance behavior of bivalves. Lysosomal response is considered as the most reliable effect observed in mussels during stress and ACP is a lysosomal marker enzyme that exists in the haemocytes and serum of bivalve. The decrease in ACP activity at the initial stages of exposure may be due to the reason that, the available enzyme must be used up in sequestering the metals that have already made entry into the cell, and also due to shell closure. A low ACP activity would show that tissue damage is less compared to those exposed to higher concentrations. Severe damage to tissues results in seepage of enzymes from cells and tissues to haemolymph resulting in high levels of enzymes in the haemolymph compartment. Acid phosphatase activity

and metallothione in synthesis are so closely related that binding of metals to the lysosomal membranes causes increased loading of metal binding proteins within the lysosomal compartment. The excess engulfing into and storing of metals in the lysosome of hemocytes and other cells lead to membrane labilisation and hydrolases are released, which are capable of cellular component lysis. Hydrolases are, therefore, predominantly sequestered in an inactive form within a thick membrane in order to prevent free access to cellular constituents. This property is termed structure-linked latency. Toxicity of metals disrupts lysosomal membrane integrity and causes its destabilization followed by release of stored lysosomal hydrolases into the haemolymph thereby increasing the activity of the enzyme in hemolymph.

Check Your Progress

14. What is clinical analysis?
15. Which electrolytes are measure in serum electrolyte test?
16. Define BUN.
17. What do we get by measuring the concentration of serum proteins?

5.6 RADIO IMMUNO ASSAY (RIA): PRINCIPLE AND APPLICATIONS

RIA is an important technique in clinical biochemistry for estimation of hormones, steroids and drugs which are present in very minute quantities and cannot be detected by the colorimetric or other reagent methods. The technique is based on the specificity of the antigen – antibody reaction.

Principle

It is a competitive binding assay in which fixed amount of antibodies and labelled antigen, i.e. conjugated to a radio-isotope (Ag*), react in the presence of the known (standards) or the unknown (samples) amounts of the antigen for binding site to the antibody (Ab). At equilibrium, the amount of radiolabelled Ag* bound to antibody will decrease as the amount of unlabelled Ag will increase.

Without competition

$12Ag^* + 3Ab \rightarrow 6Ag^*3Ab + 6Ag^*$

With competition

$12Ag^* + 3\,Ab + 6Ag \rightarrow 4Ag^*2Ab + 2Ag\,Ab + 8Ag^* + 4Ag$

Now Free Ag* and Ag* bound to antibody can be separated and quantitated by measuring the rate of emission of radiation. By using varying amounts of Ag and fixed amount of Ag*, a calibration curve can be plotted.

NOTES

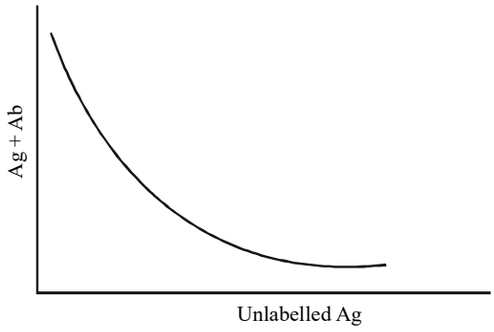


Fig. 5.14 *Calibration Curve*

Requirements and reagents

Apart from the test samples (unlabelled Ag of unknown concentrations) e.g. blood, serum, urine etc., the following are required:

1. Labelled antigen (Tracer)-High purity Ag should be used. The pure antigen is to be tagged with a radio-isotope. Usually the tyrosine in protein antigen is labelled with I^{125} either by chloramine T or by Lactoperoxidase method. Non protein antigens are labelled with tritium.
2. Antibody against the test substance should be available with high specificity, high titre and high affinity.
3. Standard Antigen (unlabelled Ag of known conc.) must be available in pure form.
4. A suitable buffer of pH 7-9.

Scintillation counter

Gamma counter for I^{125} labelled antigen. This label has very high sensitivity and low half life. Beta counter for H^3 labelled antigen. This has lower sensitivity but higher half life.

Procedure

A series of test tubes are taken in which varying amounts of pure antigen are incubated with fixed amounts of the labelled antigen and antibody for 6 hrs. The free and the bound antigen are separated and the radio-activity is counted in the bound Ag*Ab fraction. The test sample with the unknown amount of antigen is processed similarly. Known quantity of unlabelled Ag as different dilutions of standards are taken to draw a standard curve (dose response curve). By referring to the standard curve, the amount of antigen can be obtained.

Methods of separation of bound and unbound antigen

The methods of separation of bound and unbound antigens are as follows:

- Ion exchange chromatography.
- Adsorption of free antigen on charcoal followed by centrifugation leaving the bound Ag*-Ab in the supernatant.
- Using 2nd antibody against the first antibody.

- Salt precipitation of Ag* Ab complex.
- Immobilization of the antibody on a solid support and washing away of the free antigen.
- Using Protein-A of Staphylococcus aureus. This has high affinity for human IgG.

Advantages

The advantages of RIA are as follows:

- It can be used to assay any compound which is immunogenic, available in pure form and can be radio-labelled.
- It is a very sensitive test.
- It is highly specific and reproducible.
- It has a broad range of measurement.
- It can be automated.

Disadvantages

The disadvantages of RIA are as follows:

- Very high cost of equipments and reagents.
- Half life of I125 is 60 days which requires frequent ordering.
- Hazards of radioactivity.
- Lengthy procedure.

Applications

RIA is used in the measurement of peptide/steroid hormones such as T3, T4, TSH, hCG, progesterone etc in clinical chemistry.

Check Your Progress

18. What is the radioimmunoassay technique based on?
19. What happens at equilibrium in the RIA technique?
20. State the applications of RIA.

5.7 BLOOD GAS ANALYSIS

Blood gas analysis or most commonly referred as arterial blood gas (ABC_1) is an essential part of diagnosing and managing a patient's oxygenation status and acid base balance. An ABC_1 analysis evaluates how effectively the lungs are delivering O_2 to the blood and how efficiently they are eliminating CO_2 from it. The test measures the partial pressure of O_2 and CO_2 in the blood as well as O_2 content, O_2 saturation, HCO_3^- content and blood pH. Blood gas studies are performed to assess respiratory diseases, functioning of lungs and to manage patients receiving oxygen therapy.

In order to meeasure blood gases, the arterial blood is used instead of venous blood, this is because,

NOTES

- Arterial bood is a good way to sample a mixture of blood that has come from various parts of the body.
- Blood from a central venous catheter is usually an incomplete mix of venous blood blood from various parts of the body. For a sample of completely mixed blood, a sample would have to be obtained from the right ventricle or pulmonary artery. Even then, it is not necessary to know that how well the lungs are oxygenating the blood.
- Arterial blood gives the additional information of how well the lundgs are oxygenating the bloodl. If it is known that arterial O_2 concentration is normal, it indicates that the function of lungs is also normal, but if mixed venous O_2 concentration is low, it can be inferred that the heart and circulation are failing.

In case the central venous catheter blood is low in O_2 concentration, it means that the lungs have not oxygenated the arterial blood well (as a result of which venous blood has a low O_2 concentration) or the heart is not circulating the blood well (In this case, the tissues of the body must take more than normal amount of oxygen from each cardiac cycle as blood is flowing slowly. This gives rise to low venous O_2 concentration).

Obtaining arterial blood sample

Blood gas analysis is performed on blood from an artery. The patient should breathe normally during the test. Patients have no restrications on drinking or eating before the test. If the patient is receiving O_2, the O_2 concentration must remain the same for 30 minutes before the test.

Procedure

Keep the patient either in the sitting or supine position. Elevate the wrist with a small pillow and ask the patient to extend fingers downward in order to flex the wrist and move the radial artery closer to the surface. Palpate the artery and rotate the hand of the patient back and forth until a good strong pulse is left. It should be noted that for arterial puncture, the brachial and radial arteries are preferred one.

The artery to be punctured is identified by its pulstions. The skin is properly cleaned to prepare antiseptic site for puncture. For this, swab the area liberally with an antiseptic agent such as betadine. A local anaesthetic wheal may be also be made. In such a case, an anasethetic is not required. Anaesthetise the area with a small amount of 1% xylocaine (about 1/4 mL or less). This allows a second attempt without much pain, if the first attempt is failed. Make the puncure using a 20 or 21 gauge needle and then attach the preheparinised 12 mL syringe once the artery has been entered. Pull the plunger on the syringe and collect a 3 to 5 mL sample. Withdraw needle and place 4" × 4" absorbent bandage over the puncture site and maintain pressure with two fingers for atleast two minutes. In the meantime, expell any air bubbles in the blood sample as quickly as possible. The syringe should then be capped and rotated gently to mix heparin with blood. Place the sample in an ice water container, if the sample is not expected to be analysed for 15 to 20 minutes.

After the blood sample is obtained, the needle should be removed and an airtight cap placed over the lip of the syringe. At times, it is either impractical or impossible to obtain arterial blood from a patient. Under these circumstances, another source of blood can be obtained, but the most accurate results are achieved with arterial blood. The venous blood can be readily obtained, but it usually reflects the acid-base status of an extremity, not the body as a whole. Arterialised skin puncture blood from the finger has been recommended as a suitable substitute for arterial blood for pH and PCO_2, but is not acceptable for PO_2. After the blood has been taken, place the absorbent bandage over the puncture site and maintain pressure with two fingers for 5 minutes to stop blddding. The patient should rest quitely while applying pressure to the punchered site. Risks are very low when the test is done correctly. There may be bruising or delayed bleeding from the site. Very rarely there may be a problem associated with circulation in the punctured area.

Blood gas analyser

Blood gas analysers automatically or manually measure pH, PO_2 and PCO_2 of blood. The oxygen is measured with conventional amperometric membrane oxygen electrode. The CO_2 is measured by a pH glass electrode covered with a plastic membrane that allows diffusion of only gases. Chemical sensors have been developed for blood gases, electrolytes and glucose.

5.7.1 Arterial Blood Gases

Artterial blood gases, oxygen and carbondioxide are generally reported in units of partial pressure. The partial pressure of a gas in a liquid is defined as the partial pressure of that gas with which the liquid is in equilibrium. The quantity of oxygen in the blood is usually expressed as oxygen saturation, SpO_2. The oxygen saturation is a measure of the amount of oxygen in the blood that is combined with haemoglobin compared with the total amount of oxygen which could combine with haemoglobin in that blood. Oxygen saturation and oxygen content are linearly related through the oxygen capacity. The oxygen capacity depends on the amount and type of haemoglobin in the blood. The oxygen saturation refers to the oxygen carried by the haemoglobin. A very small amount of oxygen will dissolve in the plasma in physical solution. It is about 0.0003 mL of oxygen in 100 mL of plasma for each mm Hg of the PO_2. The amount of oxygen combined with haemoglobin is related with PO_2 by a curve, known as oxyhaemoglobin dissociation curve. Oxygen saturation (not the oxygen content) is used for the ordinate so that the curve may be applied to any haemoglobin concentration.

The normal oxygen pressure gradient between the alveolar air and the mean pulmonary capillary blood is about 10 mm Hg, and the gradient between the alveolar air and the end pulmonary capillary blood (pulmonary vein) is less than 1 mm Hg. These gradients may, however, be affected by a several factors. Ventilation is the process by which air moves in and out of the lungs. Gas exchange is the process by which oxygen and carbon dioxide are exchanged between alveolar air and pulmonary capillary blood. We have seen that the partial pressure of a gas in a liquid is the partial pressure of gas with which the liquid is in equilibrium. For oxygen in arterial blood, the alveolar air is not quite this gas. In fact, there is no

NOTES

single equilibrating gas. Since partial pressure of alveolar CO_2, is under control of the alveolar ventilation, the partial pressure of arterial CO_2, that is, $PaCO_2$ is considered to give direct information regarding the state of ventilation.

• Determination of oxygen saturation

The oxygen saturation (SpO_2) is the ratio of the actual oxygen content of the haemoglobin compared to the potential maximum oxygen carrying capacity of the haemoglobin. The percentage of SpO_2 saturation is a measure of the relationship between oxygen and haemoglobin. Normal values for arterial blood saturation, SpO_2 and mixed venous blood saturation $savO_2$ are 95 percent (or higher) and 75 percent, respectively. It should be noted that the percentage of saturation does not mean the oxygen content of the arterial blood. The oxygen capacity is the maximum amount of oxygen available that can be combined with haemoglobin. Tissue oxygenation or the amount of oxygen available to the tissue can be indicated by the combined measurements of oxygen saturation partial pressure of oxygen that is PO_2 and of haemoglobin.

Procedure

Two methods can be used for the determination of oxygen saturation in an arterial blood sample.

- The arterial blood sample is introduced into the oximeter, which is a photoelectric device, and oxygen saturaion is determined directly. The value of oxygen saturaion is measured with the oximeter by making use of the principles of spectrometry.
- The oxygen content and oxygen capacity are first determined and then the oxygen saturation is determined by using the following relationship.

$$\text{Percent oxygen saturation} = \frac{\text{Oxygen content volume \%} \times 100}{\text{Oxygen capacity volume \%}}$$

In other words,

$$\text{Percent saturation} = \frac{\text{Volume of } O_2 \text{ actually combined with haemoglobin} \times 100}{\text{Volume of } O_2 \text{ with which haemoglobin may actually combine}}$$

The oxygen content of blood sample can be measured before and after exposure to atmosphere.

• Determination of oxygen (O_2) content

Oxygen content is the actual amount of oxygen in the blood. Blood may contain less quantity that it is capable of carrying. About 98 percent of all oxygen delivered to the tissues is transported in chemical combination with haemoglobin. It should be noted that 1g of haemoglobin is capable of carrying upto 0.3 mL of oxygen. This measurement can be obtained by multiplying the number of grams of haemoglobin in 100 mL of blood by 1.34 times the partial pressure of oxygen in the blood PO_2.

The normal value of oxygen content of arterial blood and that of venous blood are 15-22 vol% and 11-16 vol% respectively. Here vol% indicates volume percent, that is mL per 100 mL of blood.

NOTES

Procedure

An arterial or venous blood sample is first obtained and then the oxygen content in the blood sample can be calculated by using the following relation.

$$\text{Oxygen } (O_2) \text{ content} = SaO_2 \times \text{Haemoglobin} \times 1.34 + PaO_2 \times 0.003$$

A decrease in arterial blood oxygen, which is associated with an increase in arterial blood carbon dioxide, may be due to various reasons which include chronic obstructive lung disease, respiratory complications postoperatively, kyphoscoliosis, neuromuscular impairment and obsesity hypoventilation etc.

• Determination of partial pressure of oxygen (PO_2)

The normal value of partial pressure of arterial blood, PaO_2 is 80 torr or greater and that of venous blood, that is PvO_2 is 30-40 torr.

Oxygen is carried in the blood with haemoglobin in dissolved as well as combined form. Most of the oxygen in the blood is carried by haemoglobin. It is the partial pressure of a gas that determines the force that the gas exerts in an attempt to diffuse though the pulmonary membrane. The partial pressure of oxygen, PO_2 is the quantity of O_2 passing from the pulmonary alveoli into the blood. PO_2 is directly influenced by the quantity of oxygen being inhaled.

PO_2 is a measure of the pressure exerted by the amount of oxygen dissolved in blood plasma. This test measures the functioning or effectiveness of the lungs to oxygenate the blood.

Procedure

Obtain an arterial blood sample and then introduce a small quantity of the blood in a blood gas analyser and partial pressure of O_2 is measured by making use of polarographic Clark electrode.

Increased levels of PO_2 are generally associated with polycythaemia, hyperventilation in the arterial blood sample etc. Decreased levels are associated with anemia, insufficient atmospheric oxygen, chronic obstructive disease, cardiac decompensation, restrictive pulmonary disease and hypoventilation because of neuromuscular disease.

• Determination of carbondioxide (CO_2) content or total carbon dioxide (TCO_2)

The normal value of TCO_2 is 24-30 mEq/Litre. In normal blood plasma more than 95 percent of the total CO_2 content in contributed by bicarbonate (HCO_3), which is regulated by kidneys. The remaining 5 percent of the carbondioxide is contributed by the dissolved CO_2 gas and carbonic acid, H_2CO_3. Dissolved CO_2 gas contributes very little to the total CO_2 content, because it is regulated by lungs. The test is a general measure of the alkalinity, or acidity of venous, arterial, or capillary blood. The test is a measure of CO_2 from,

Dissolved CO_2, Total, H_2CO_3, HCO_3^-, Carbamino carbondioxide

$$\text{Total } CO_2 = HCO_3^- + 0.03 \times PCO_2$$

NOTES

Procedure

6 mL sample of venous or arterial blood is first collected in a heparinised syringe. Place the syringe in ice water, if it is not possible to analyse the sample immediately.

It should be noted that HCO_3^- in the extracellular spaces exists first as CO_2, then as H_2CO_3, and then much of it is converted to $NaHCO_3$ by the buffers of plasma and RBCs. Increased CO_2 content levels generally occur in severe vomiting, emplysema and aldosteronism. Decreased CO_2 content levels are generally associated with severe diarhoea, starvation, acute renal failure, diabetic acidosis and salicylate toxicity. In diabetic acidosis the supply of ketoacids exceeds the demands of the cell. The blood plasma acids rise. The blood plasma HCO_3^- decreases as it is used in neutralising these acids.

5.7.2 Determination of Blood pH

Normal value of venous blood pH lies between 7.32 and 7.43 and that of arterial blood between 7.35 and 7.45. The pH is the negative logarithm of the hydrogen ion concentration in the blood. The sources of H^+ in the blood are volatile acids and non-volatile acids. The non-volatile acids can not be volatilised and are fixed. Dietary acids, lactic acid and ketoacids are the examples of non-volatile acids.

The pH measurement is a ratio of acids to bases and it indicates the chemical balance in the body. The measurement of pH of blood gives an idea if the body is too acidic or too alkaline, the acid-alkali balance is extremely intricate and necessary to be kept within the slight margin of 7.35 to 7.45 pH in the extracellular fluid. The repiratory response to changes in blood pH is almost instantaneous. In acidosis, CO_2 is retained and pH decreases and it stimulates ventilation. In alkalosis, CO_2 is blown off and pH rises. This causes ventilation to depress. The pH of blood can be determined by direct as well as indirect method. In the direct method, an arterial blood sample is first obtained and a small amount of the sample is introduced into a blood gas analyser and pH is measured. In indirect method, Henderson-Hasselbatch equation is used to determine pH of blood.

$$pH = pK + \log \frac{(HCO_3^-) \text{ Major Blood Base}}{(H_2CO_3^-) \text{ Major Blood Acid}}$$

The pH gets decrease in acidemia due to increase in the formation of acids. The pH gets increased in alkalemia because of loss of acids. Metabolic acidemia is associated with renal failure, ketonacidosis in starvation and diabetes, lactric acidosis and strenuous exercise. Metabolic alkalemia is associated with deficient potassium hypochloremia, vomiting, sodium bicarbonate administration, aspirin intoxication etc. Respiratory alkalemia is associated with acute pulmonary disease, myocardial infaction, chronic and acute heart failure, anxiety, neurosis, psychoses, anaemia, CO poisoning, pain and shock etc. Respiratory acidemia is generally associated with ventilatory failure and acute repiratory distress syndrome.

Acidosis usually increase respiration whereas alkalosis usually decreases respiration.

Check Your Progress

21. Define blood gas analysis.
22. What is normal pH value of blood?
23. What does the pH measurement of blood indicat

5.8 TRACE ELEMENTS IN THE BODY

The elements which are required in very small amounts are called trace elements. These include copper, zinc, cabalt, manganese, molybdenum, iodine and fluorine. There are subdivided into three categories:

- **Essential trace elements:** These include iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium.
- **Possibly trace elements:** There include nickel, vanadium, cardmium and barium.
- **Non-essential trace elements:** These include aluminium, lead, mercury, boron, silver, etc.

Some of these trace elements are as follows:

- **Calcium:** It is the most abundant metal found in the body. It constitutes about 2% of the body weight. About 99% calcium is present in the bones and teeth. The important biochemical functions of the calcium in the body include:
  - (i) **Development of bones and teeth:** Calcium and phosphorus are the two most important non-protein body building materials. Calcium exists in the bones as the double salt of calcium and phosphate, $CaCO_3.nCa_3(PO_4)_2$, where n varies from 2 to 3. Calcium, alongwith phosphate is required for the formation of hydroxyapatite and physical strength of sketetal tissue. Bones act as reservoirs of calcium.
  - (ii) **Muscle contraction:** Ca^{2+} interact with troponin C to trigger muscle contraction. Ca^{2+} ions neutralise the negative charge of myosin which then reacts with negatively charged actin as a result of which, contraction takes place. In this way, calcium increases the interaction between actin and myosin. Ca^{2+} ions also activate the myosin ATPase, which in turn breaks down the ATP to provide energy required for contraction.
  - (iii) **Blood coagulation:** Various reactions in the cascade of blood clotting process depend on Ca^{2+} ions. Blood contains about 9-11.5 mg/100 mL of calcium. Of this, very small amount is present in red blood cells (RBC) and most of the calcium is confined to plasma.
  - (iv) **Nerve transmission:** Ba^{2+} ions are necessary for the transmission of nerve impulse. Neuromuscular transmission is the process of transmission of nerve impulse from the nerve to a muscle across the neuromuscular junction. Acetyl choline acts as neurohuman transmitter and Ca^{2+} ions help in the release of acetyl choline.

(v) **Membrane integrity and permeability:** Ca^{2+} ion influences the membrane structure and transport of water and several ions across it. Generally membrane permeability is decreased by calcium and this effect balances the opposite action of sodium and potassium capillary permeability.

(vi) **Activation of enzymes:** Ca^{2+} ions are required for the direct activation of various enzymes which include lipase, adenosine triphosphate, ATPase and succinate dehydrogenase.

(vii) **Release of hormones:** Ca^{2+} ions also facilitate the release of certain hormones such as insulin, PTH, calcitonin etc., from the endocrine glands. PTH = Parathyroid hormone.

(viii) **Secretory processes:** Ca^{2+} also regulates microfilament and microtubule mediated processes such as endocytosis, exocytosis and cell motility.

(ix) **Calcium as intracellular messenger:** There are certain hormones which exert their action through the mediation of Ca^{2+} ions. Calcium is regarded as a second messenger for such hormonal action, e.g., epinephrine in liver glucogenolysis. Calcium also acts as a third messenger for some hormones, e.g., antidiuretic hormone (ADH) acts through cAMP and Ca^{2+}.

(x) **Calmodulin mediated action:** Calmodulin is a calcium binding regulatory protien. The calcium-calmodium complex activates certain enzymes, e.g., adenylate cyclase, Ca^{2+} dependent protien kinases.

(xi) **Contact inhibition:** Calcium is believed to be involved into cell to cell contact and adhesion of cells in a tissue. Ca^{2+} may be required into cell to cell communication.

(xii) **Excitability of nerves:** Calcium is essential for the excitation of nerves.

(xiii) **Calcium in growth:** There cannot be growth without bone development. Calcium is essential and directly as well as intimately related with growth.

- **Magnesium:** The body of an adult contains about 20g magnesium, 70% of which is found in bones in combination with calcium and phosphorous. The remaining 30% of magnesium is found in soft tissues and body fluids. The important biochemical functions of magnesium include.

  (i) Magnesium is required for the formation of bones and teeth. Mg^{2+} ions serve as a cofactor for various enzymes requiring ATP, e.g., hexokinase, glucokinase, phosphofructokinase and adenylate cyclase. Mg^{2+} also act as activator of other enzymes such as phosphorylase, enolase, peptidase, alkaline, phosphatase, RNA polymerase, DNA polymerase and many others.

  (ii) Mg^{2+} ion is necessary for proper neuromuscular function. Neuromuscular irritability is caused by the deficiency of Mg^{2+}. In the body, Mg and Ca act as antagonists to one another and counter-act certain effects of one another. For example, the depression of the central peripheral nervous system due to hypermagnesium can be prompty reversed by intravenous admistration of calcium.

(iii) Like calcium, magnesium is excreted in the faeces and urine. Under normal conditions about 50-80 percent of magnesium is exerted in the faeces and remaining by the kidneys.

(iv) Magnesium is absorbed by the intestinal cells through a specific carrier system. Generally about 50% of the dietary magnesium is absorbed. The absorption of magnesium decreases by the consumption of large amounts of calcium, phosphate and alcohol. Mg absorption is, however, increased by PTH.

(v) Magnesium dificiency generally causes nueromuscular irritation, weakness and convulsions.

- **Sodium:** Sodium is the main cation in the extracellular fluid. About 50% of body sodium is present in the bones, 40% in the extracellular fluid and remaining 10% in the soft tissues. Sodium requirement for an adult is 4-5 g/day and for a patient of hypertension about 1g/1day. Main biochemical functions of sodium include:

  (i) Sodium regulates the acid-base balance of the body in association with chlorine and carbonate.

  (ii) Sodium is required for the maintenance of osmotic pressure and fluid balance. In fact, the primary function of sodium, potassium and chloride ions in the body is to maintain the normal osmotic pressure of the different body fluids throughout the body. In this way, these ions protect the body against exessive loss of fluids, which may otherwise disturb the normal hydration. Sodium, being the chief cation of extracellular fluid, plays an important role in this regard, because osmotic pressure of a fluid depends upon the total cations of that fluid.

  (iii) Sodium is involved in the intestinal absorption of glucose, galactose and amino acids.

  (iv) Sodium is also necessary for initiating and maintaining heart beat. In blood plasma, sodium and potassium chlorides have the most important function not only in keeping the gloubulins in physical solution, but also in regulating the degree of hydration of the plasma proteins. These are necessary in the maintenance of the proper viscosity of the blood.

  (v) Sodium is readily absorbed in the gastrointestinal tract and hence very little of it is found in faeces. However, large amount of sodium is lost in faeces in diarrhoea. Kidney is the major route of excretion of sodium from the body. Extreme sweating also causes significant amount of sodium loss from the body. There is, however, individual variation in sodium loss through sweat.

- **Potassium:** Potassium is the principal intracellular cation. For specific functions, it is very important in the extracellular fluid, because it maintains intracellular osmotic pressure. Potassium is required for the regulation of acid base balance and water balance in the cells. The sodium and potassium salts with the corresponding weak acids from the chief buffer systems respectively of extracellular and intracellular fluids. These buffer systems play vital roles in the regulation of pH of various fluids under various physiological conditions.

Potassium is also required for the transmission of nerve impulse. Extracellular K^+ ion influences cardiac muscle activity. The optimal activity of the enzyme pyruvate kinase of glycolysis depends on K^+ ion. Adequate intracellular concentration of K^+ ion is necessary for proper biosynthesis of proteins by ribosomes. Potassium goes into the intracellular fluid during the period of cell growth and repair. The absorption of K^+ ion from the gastrointestinal tract is very efficient and so very little is lost through faeces. A good amount of K^+ is, however, lost in subjects with diarrhoea. Potassium is normally excreted almost entirely by the kidneys in the urine K^+ excretion is, however, influenced by the maintenance of acid base balance. Excretion of potassium is enhanced by aldosterone. An adult man requires about 3-4g per day to potassium is found in almost all foods. The good sources of potassium are banana, orange, pineapple, potato, beans, chicken, liver, coffee, tea, milk, tender coconut water etc.

- **Copper:** Copper is essential to life and adult humans contain about 100 mg of Cu. About 4-5 mg of Cu is required daily in the diet, longer amount of Cu is however toxic. Dietary sources of copper include coconut, almonds, nuts, papayas, oranges, grapes and vegetables. Main biochemical functions of copper include:
  (i) In the body, copper is bound to protiens either as metalloprotiens or as enzymes. Various oxidases and blue protiens are the examples, which include

  (a) Amine oxidases (oxidation of amines), (b) Ascorbate oxidase (oxidation of ascorbic acid), (c) Cytochrome oxidase that acts with haem as the terminal oxidase step, (d) Galactose oxidase (oxidation of an OH group to CHO in the monosaccharide galactose.

  (ii) Cooper is also important in

  (a) Lysine oxidase, which affects the elasticity of aortic walls, (b) Dopamine hydroxylase, which affects brain function, (c) Tyrosinase, which affects skin pigmentation, (d) Ceruloplasmin, which plays important role in Fe metabolism.

  (iii) Copper is an essential constituent of several enzymes such as cytochrome oxidase, catalase, tyrosinase, super oxide dismutase, monoamine oxidase, ascorbic acid oxidase, phenol oxidase and uricase.

  (iv) Copper is involved in many metabolic reactions because of the fact that it is present in a wide variety of enzymes.

  (v) Copper is necessary for the synthesis of haemoglobin because copper is a constituent of ALA synthesis.

  (vi) Copper is essential for the synthesis of vitamin and phospholiphids.
- **Zinc:** Biologically, zinc is the second most important transition metal. It plays an important role in several enzymes. Zinc content in serum, tissues is 1-2 ppm, 12-100 ppm and 0.3-0.6 mg/day respectively. Dietary sources of zinc include cereals, nuts, oil seeds, grains, soyabeans, wheat, peas, potatoes, onion, almonds etc. Zinc requirement for an adult is 10-15 mg/day. Biochemical functions of zinc include:

(i) Zinc is the essential component of various enzymes such as alkaline phosphotase, carboxy-peptidase, alcohal dehydrogenase, carbonic analydrase and superoxide dismutase.

(ii) Zinc is required for maintaining normal levels of vitamin A in serum. It is needed to transport vitamin A to the retina and this improves vision.

(iii) Zinc is a crucial nutrient for immune function, healing nervous system, brain function, blood sugar, balanced reproduction and optimal health.

(iv) Gusten, a zinc containing protein is important for taste sensation.

(v) Deficiency of zinc is associated with neuropychiatric disorders including anorexia, nervous depression, schizophrenia and alzheimer disease.

- **Molybdenum:** It is an essential constituent of certain metalloflavour proteins enzymes such as xanthine oxidase, aldehyde oxidase, nitrate reductase, sulphite oxidase and hydrogenase. Mo is absorbed in intestine and is very little retention of Mo, except in the bones and also in liver and kidneys, but is very small amounts.
- **Manganese:** The total body content of Mn is about 15 mg. Most of the manganese in the body is concentrated in liver, muscles, kidneys and bones, but liver and kidney are rich in Mn. Blood contains about 4 to 20 μg Mn per 100 mL, about 2/3rd of which is in the cells. Within the cells, Mn is mainly found in the nuclei in association with nucleic acids.

  (i) Manganese serves as a cofactor for several enzymes which include arginase, pyruvate carboxylase, isocitrate dehydrogenase, superoxide dismutase and peptidase, Mn as Mn^{2+} activates a number of enzymes such as liver arginase, phosphoglucomutase, mitochondrial respiratory enzyme system and choline esterase.

  (ii) Mn(+2) is important in both animal and plant enzymes. In mammals, the enzyme arginase is produced in the liver and this is important because it converts nitrogenous waste products into urea in the ornithine-arginine-citrulline cycle. The urea is carried by the kidneys, where it is exerted in urine.

  (iii) Mn is an essential trace element for plant growth. It is added to fertilizers where there is a deficiency in the soil.

  (vi) It is essential for a group of enzymes called phosphotransferases.

- **Iron:** About 70% of the total iron present in the body occurs in erythrocytes of blood as a constituent of blood. About 5% of body iron is prsent in myoglobin of muscles. Major dietary sources of iron include liver, meat, fish, poultry, cereals, legumes, nuts, oilsseeds, dry fruits, leafy vegetables etc. Iron requirement for an adult and pregnant woman are 10-14 mg/day and 40 mg/day respectively. An adult body contains about 3g of iron. Major biochemical functions of iron include:

  (i) Heme is the most predominant iron containing substance and it is a constituent of several proteins and/or enzymes. Hermoproteins include haemoglobin, myoglobin, cytochromes, catalase, xanthine oxidase, tryptophan pyrrolase and peroxidase. There are certain proteins which also contain non-heme iron. Examples include transferrin, ferrin and hemosiderin.

NOTES

Iron mainly exerts its functions through the compounds in which it is present. Haemoglobin and myoglobin are required for the transport of oxygen and carbon dioxide. Iron is an essential constituent of haemoglobin, which carries oxygen from lungs to other tissues. The muscles store oxygen in combination with myoglobin, which also contains iron.

(ii) Cytochromes and certain heme proteins are necessary for electron transport chain and oxidative phosphorylation. Iron forms an integral part of all the cytochromes as well as certain other enzymes such as catalase and peroxidases. Cytochromes are responsible for catalysing biological oxidation and providing energy. Iron also forms a part of prosthetic group of some othe flavoproteins and so involved in electron transfer.

(iii) Peroxidase, the lysosomal enzyme is required for phagocytosis and killing of bacteria by neutrophilis. Iron is also associated with effective immunocompetence of the body.

(iv) In hemosiderosis, excessive iron is deposited in ferritin and hemosiderin.

(v) In hemochromatosis, iron is directly deposited in tissues (linear, spleen, pancreas) causing bronze diabetes.

(vi) Iron deficiency causes anaemia. The various factors responsible for this disorder include inadequate or defective absorption of iron, chromic blood loss.

- **Iodine:** Human body contains about 20 mg of I_2. About 80% I_2 is stored in the form of iodothyroglobutin in the thyroid gland. Richest sources of iodine are sea foods, shell fish, fish oil, fruits, vegetables, cereals, milk, meat, eggs and iodized salt. Requirement of iodine far adults, women and children are 100-150 μg, 150 μg and 80 μg respectively important functions of I_2 are:

  (i) I_2 is required for the synthesis of thyroid harmones such as thyroxin and tri-iodothyroxine. Thyroxin controls metabolism, utilization of sugars, regulates energy production and aids growth. It improves cognition and makes skin, hair and teeth healthier.

  (ii) Deficiency of I2 may cause cretinism in children. Dwarf child is mentally retarded with enlarged thyroid gland.

- **Phosphorus:** It is essential for life, both as a structural material in higher animals, and in the essential metabolism of both plants and animals. An adult body contain about 1 kg phosphate and it is found in every cell of the body. About 80% of phosphorus is found in bones and teeth in combination of Calcium. 10% P is found in muscles and blood. The remaining 10% is widely distributed in various chemical compounds. Important functions of P includes:

  (i) It is most essential for the development of bones and teeth.

  (ii) It plays an central role in the formation and utilization of high energy phosphate compounds such as ATP, GTP and creatine phosphate etc.

  (iii) It is required for the formation of phospholipids, phosphoproteins and nucleic acids such as DNA and RNA.

(iv) Phosphrus is an essential component of several nucleatide coenzymes such as NAD^+, $NADP^+$, ADP, AMP and pyridoxal phosphate.

(v) Phosphate is also necessary for the absorption and metabolism of carbohydrates in the body.

- **Fluorine:** Fluorine is found in small quantities in normal bones and teeth. It performs following functions:
  (i) Traces of F^- in drinking water (1 ppm) greatly reduce the incidence of dental carries (too decay).
  (ii) F^- ions make the enamel on teeth much harder.
  (iii) Concentration of F^- above 2 ppm, however may cause discolouration, the brown mottling of teeth.
  (iv) The fluorine requirements of the body are met by the quantity normally present in drinking in most regions. Too much fluorine in diet can, however, be harmful.

Check Your Progress

24. Define trace elements.
25. Give examples of some essential trace elements.
26. What is hyponatremia?

5.9 DRUG ANALYSIS

A drug is any chemical substance that causes a change in an organism's physiology or psychology when consumed. Drugs are typically distinguished from food and substances that provide nutritional support. Consumption of drugs can be via inhalation, injection, smoking, ingestion, absorption or dissolution under the tongue.

In pharmacology, a drug is a chemical substance, generally of known structure, which when administered to a living organism, produces a biological committee. A pharmaceutical drug or generally referred as medicine is a chemical substance used to treat, cure, present, or diagnose a disease or to promote well being. Traditionally drugs were obtained through extraction from medicinal plants, but more recently also by organic synthesis. Pharmaceutical drug may be used for a limited duration on a regular basis for chronic disorder.

5.9.1 Classification of Drugs

Various drugs can be classified in following modes:

1. **On the basis of pharmacological effect:** According to pharmacological classification, drugs are classified depending on their action on an organism (the heart, brain, lymphatic system stomach, intestines etc.) For example, an analgesic reduces pain while an anti-inflammatory drug reduces the inflammation of the body. Accordingly, drugs are classified into groups such as narcotics, soporifics, analgesics, diuretics, and local anaesthetics.
2. **On the basis of chemical structure:** This classification is based on the chemical structure, properties, regardless of their pharmacological action.

NOTES

For example, the group of cardiac stimulants include compounds from heterocyclic series such as caffeine, strychnine pantoprazole, terpenes (camphor) and cardia glycosides (steroids).

3. **One the basis of their effects:** According to their effects, drugs can be classified into the following groups:

   A. Anti-infective drugs: The drugs interfere selectively with the functioning of microorganisms while leaving the host unharvest. These are of following types:

   (i) Antibiotics srugs such as sulpha drugs, penicillins, cephalosporins etc., either kill bacteria directly or prevent them from multiplying so that the body immune system can destroy invading bacteria.

   (ii) Antifungal drugs can cure or may only suppress a fungal infection.

   (iii) Antiviral drugs prevent the penetration of virus into host cell or block the synthesis of new viruses. With some viruses such as HIV which cause AIDS, antiviral drugs can only prolong life but can not cure the disease. Vaccines are used as antiviral drugs against poliomyelitis, influenza and mumps, etc.

   B. Endocrine drugs: These affects the hyperactivity and hypo activity of body's natural hormones. For example:

   (i) Progesterone or progestin hormone is used for the osteoporosis and atherosclerosis.

   (ii) Estrogen and progesterone are used in birth control pills.

   (iii) Insulin is employed to treat diabetes.

   (iv) Androgen is used to relieve from hot flashes and mood swings.

   C. Central nervous system drugs (CNS): CNS drugs are used to treat neurological and psychiatric problems. These include:

   (i) Antianxiety drugs or tranquilizers treat anxiety centres of the brain. Tranquilizers such as benzodiazepines, diazepam, chlordiazepoxide, meprobamate are used as anxiolytics, anaesthetics and anticonvulsants.

   (ii) Antimanic drugs like lithium dampens extreme mood swings in patients. These are used to treat manic depressive (extreme excitement and lethargy, i.e., bipolar nature) disorders.

   (iii) Antidepressant drugs alleviate mental depression. These drugs include pargyline, amitriptyilne, and sertaline.

   (iv) Antipsychotic drugs regulate certain brain mechanisms called neurotransmitters which do not function properly in people with psychosis, hallucinations or major mental disorders.

   (v) Analgesic drugs: Narcotics such as codeine, morphine, heroine, meperidine relieve pain by acting on receptors located on the nerve cells of brain or spinal cord. Non narcotic analgesics like ibuprofen, aspirin, acetaminophen reduce pain or fever by inhibiting the formation of nerve impulses at the site of pain.

(vi) Sedatives are CNS depressants that are capable of reducing nervous tension and promote relaxation without producing sleep. Simple bromides act as good sedative with no hypnotic action

(vii) Stimulants like caffeine, nicotine, ephedrine increase neuronal activities, reduce fatigue and appetite

(viii) Psychomotor stimulants such as cocaine and methamphetamine stimulate sensory motor functioning and are used to treat attention deficit hyperactivity disorder (ADHD) and narcolepsy.

(ix) Stimulatory hallucinogenic produce a mixture of psychomotor stimulant and hallucinogenic effects.

(x) General anaesthetics depress brain activity to such an extent that all sensitivity to pain is lost thus causing unconsciousness during surgery.

(xi) Local anaesthetics like novocaine, dicaine, benzocaine make a particular organ insensitive. They prevent nerves from transmitting impulses signalling pain.

D. Cardiovascular drugs: These drugs affects heart and blood vessels. These include:

(i) Antihypertensive drugs like losacar, loram, amlopin reduce blood pressure by dilating blood vessels and reduce the amount of blood pumped by the heart into the vascular system.

(ii) Antiarrhythmic drugs normalise irregular heart beats prevent cardiac malfunction.

E. On the basis of therapeutic action: These can be further classified as:

(i) Chemotherapeutic Drugs: These drugs are used to cure specific diseases such as malaria, sphilis and tuberculosis etc. Chemotherapeutic drugs include antibiotics, antiseptics, antineoplastic etc.

(ii) Pharmacodynamic drugs assist in the recovery from specific bacteria or -viral infection. These drugs include tranquilizers, anaesthetics, antipyretic, antihistamines etc.

F. On the basis of effect on blood: These can be further classified as:

(i) Antianaemic drugs such as iron capsules and vitamins enhance the formation of red blood cells.

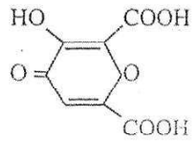
(ii) Anticoagulants like heparin reduces blood clot formation to ensure free blood flow in the body

(iii) Thrombolytic drugs: Dissolve blood clots, which can block blood vessels leading to heart stroke.

5.9.2 Narcotics

Narcotics is a drug that produces analgesia (pain relief), narcosis (state of stupor or sleep) and addiction (physical dependence on the drug). Sometimes, narcotics

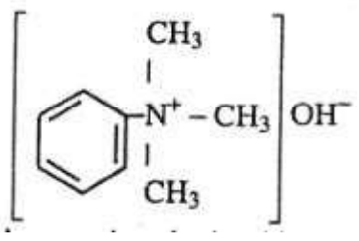
also produce euphoria (a feeling of great elation). The main therapeutic is for pain relief and hence they are often called narcotic analgesics. In recent years, narcotics are used as pain relievers, cough suppressants and to control diarrhoea. Natural narcotics are those which are found in opium. Examples of such narcotics are morphine and codeine. Both morphine and codeine are the main alkaloids of phenanthrene isoquinoline derivatives. Both are contained in opium produced from the opium poppy Papaver somniferous. The alkaloids are contained in the opium in the form of salts of meconic acid,



Meconic acid

Some of the narcotics drugs are as follows:

- **Morphine:** It is a drug that strongly alleviates pain, and is used during post operative period to patients suffering from malignant tumours and other severe diseases with great pain. It causes pleasant feeling and drowsiness. The repeated taking of morphine causes drug addiction.
- **Codeine:** It is produced for medical purposes from morphine by its methylation, using a semi-synthetic process. Trimethylphenylammonium hydroxide has been found to be the best methylating agent.



Trimethylphenylammonium hydroxide (a quaternary base)

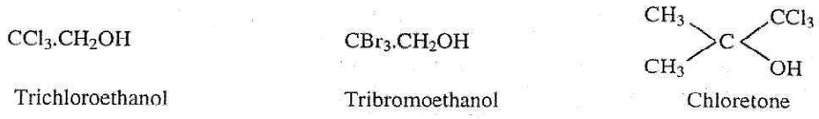
The analgesic (narcotic) action of codeine is considered to be weaker than that of morphine. Codeine does not cause drug addiction in small doses and is much less toxic. However, codeine in large doses and with prolonged administration may cause addiction.

- **Semi-synthetic drugs:** The narcotics derived from morphine, codeine and thebaine are known as semi-synthetic drugs. They include heroine, hydrocodone, hydromorphone and oxycodone.
- **Sedatives** are CNS depressant that are capable of reducing nervous tension and promote relaxation without producing sleep.
- **Hypnotic**s are CNS depressants that produce sleep resembling natural sleep. The commonly used sedatives or hypnotics may be one of the following groups of compounds.

- **Alcohols:** Ethyl alcohol was used as sedative and hypnotic for a long time. But it was replaced by other alcohols such as amyl alcohol because continuous use of ethyl alcohol leads to alcoholism. The halogen derivatives of alcohol have also been used for the same purpose. As regards its pharmacological properties, ethyl alcohol belongs to substances with a narcotic action. By acting on the cerebral cortex, it produces a typical alcoholic excitation, while in large doses it weakens the stimulating process in the cortex and suppresses the activity of the respiratory centre.

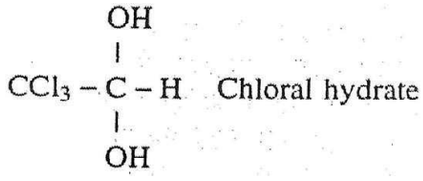
  The hypnotic activity of alcohols increases with increase in molecular weight and branching of the alkyl group. The sedative property of alcohols decreases in the order: tertiary > secondary > primary. The hypnotic activity of the alcohol also increases by replacing the hydrogen by a halogen.

  Among the halogenated alcohols, trichloroethanol, tribromoethanol and chloretone have been used as sedatives and/or hypnotics.



- **Aldehydes:** The physiological action of drugs containing an aldehyde group in their molecule varies and depends mainly on the nature of the alkyl radical bound to the carbonyl carbon. An increase in the length of the alkyl radical in an aldehyde molecule amplifies its physiological action or activity, but its toxicity also increases.

  The introduction of a halogen into an aldehyde molecule increases its narcotic or sedative action. Thus narcotic properties of chloral are more pronounced than those of acetaldehyde. An aldehyde group increases the toxicity of a substance, but it can be substantially decreased by the formation of the hydrate form of the aldehyde. Only a chlorinated aldehyde can form a hydrate. The hydrate form of chloral has a low toxicity and is used in medicine as chloral hydrate exhibiting a sedative action.

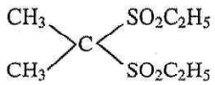


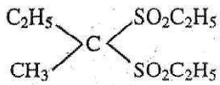
  In small doses, chloral hydrate causes a sedative effect, but in large doses it induces a deep sleep. Butyl chloral hydrate has also been found to have sedative action, but the hypnotic action of butyl chloral hydrate is less powerful than the chloral.

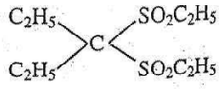
- **Ketones:** Most of the ketones have hypnotic properties. For example, diethyl ketone, $C_2H_5COC_2H_5$ is a strong hypnotic, but it could not be used longer due to its insolubility and bitter taste. Benzo-phenone also have a slight hypnotic action. The mixed aromatic and aliphatic ketones, such as acetophenone, $C_6H_5COCH_3$ are fairly strong hypnotic. Acetophenone is used under the name hypnone. The hypnotic properties further increases by

introducing the ethyl group in the molecule. Thus phenyl ethyl ketone. $C_6H_5COC_4H_5$ is more powerful hypnotic than acetophenone.

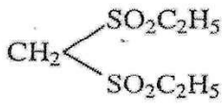
- **Sulphones:** Sulphonal has been found to have strong hypnotic action on animals. Increase in the number of ethyl groups has been found to increase the hypnotic action. Thus trional is more active than sulphonal and tetronal is more active than trional.

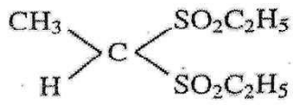

Sulphonal


Trional

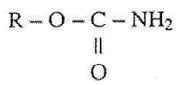

Tetronal

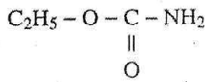
The hypnotic action of sulphones decreases by decrease in the number of ethyl groups. Moreover, only those sulphones have been found to be active as hypnotic in which two SO_2R groups are attached to the same carbon atom. The hypnotic properties also decrease by an increase in length or size of one or more of the sulphone groups.

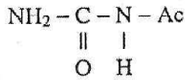

Methylene diethylsulphone (Inactive)


Ethylidenediethyl sulphone (Active)

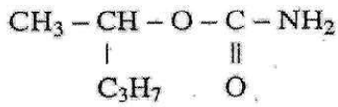
- **Urethanes:** These are the esters of carbamic acid having soporific action and are of importance in medicines. The acetyl derivatives of area are called uricides.

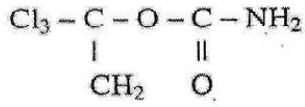

Urethane

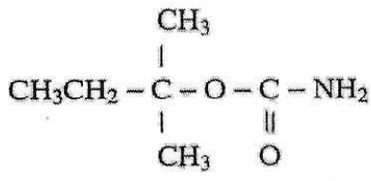

Ethyl carbamate


Ureide

Although urethane, $C_2H_5OCONH_2$ itself is not an important narcotic for man, its analogues have successfully been used as sedatives.

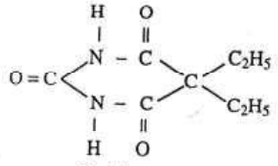

Hedonal


Voluntal

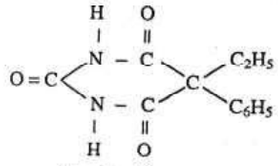

Anonal

The amide and urea derivatives are not so powerful in producing hypnotic action and thus they are mainly used as sedatives groups.

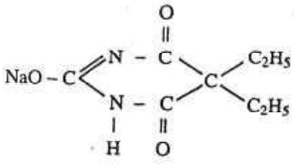
- **Barbiturates:** When the hydrogens in the methylene group of barbituric acid substituted by various radicals, barbiturates are obtained and these are used in medicine as soporific drugs. Some important drugs of barbiturate group are given below:



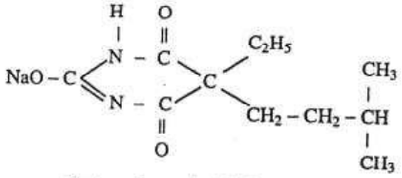
Barbitone



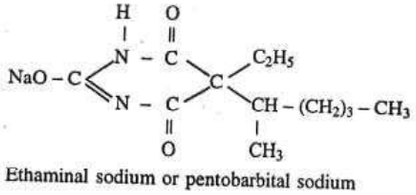
Phenobarbitone



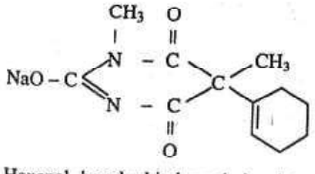
Barbital sodium



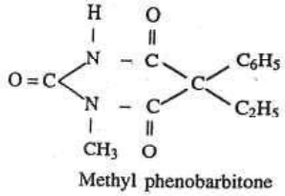
Barbamyl, amobarbital, pentymal



Ethaminal sodium or pentobarbital sodium



Hexenal, hexobarbital, methylcyclobarbital



Methyl phenobarbitone

In this way, a very large number of therapeutically active drugs have been developed, chiefly with a soporific action. Some barbiturates, in addition to their soporific effect, exhibit an anti-convulsive action (e.g., phenobarbital) while others are administered as narcotics (e.g. hexobarbital or hexenal). Phenobarbitone can be used as sedative as well as hypnotic.

- **Tranquilizers:** These are the drugs which are used in the treatment of mental disorders. These drugs produce specific improvement in the mood and behaviour of patients suffering from mental disorder. Tranquilizers cause sedative action without producing sleep. Tranquilizers are also known as antipsychotics or neuroleptics, because they suppress mania and psychotic overactivity. Tranquilizers include antipsychotics (or neuroleptics), anti-anxiety agents, central nervous system stimulants or antidepressants and psychotogenic drugs.

5.9.3 Dangerous Drugs

Dangerous drugs exert adverse side effects on the various body organs of the addict. These illegal drugs have the following effects:

- Psychotogenic drugs produce psychosis, depersonalisation, changes in mood, behaviour and retarded memory.
- Hallucinogens or psychotomimetics like marijuana, peyote and LSD cause alterations in normal thoughts, perceptions and moods.

NOTES

- Overdoses of barbiturates may cause poisoning, respiratory failure and death.
- Excess of caffeine can result in exhaustation of nerve cells.
- Iproniazide is a toxic drug which may produce necrosis of liver.
- Cannabis or marijuana, hashish, charas ganja are dangerous abuse drugs which may induce mutation damage to chromosomes and disrupt the growth of faetus.
- Mascaline, a psychotogenic drug, has mutagenic and teratogenic effects.

5.9.4 Screening of Drugs: Gas and Thin-Layer Chromatography

Gas chromatography has been employed successfully in pharmaceutical analysis. This technique is specially effective when analysing volatile substances contained in small amounts. It is best suited for analysing numerous drug samples in blood, urine and other body fluids. Screening procedure in-value following steps:

1. **Solvent extraction prior to drug isolation:** Liquid-Liquid solvent extraction is used to separate drugs from biological fluids and pre-concentrate them before GC measurement.
   - Different classes of drugs can be extracted from an aqueous solution at different pH into a solvent such as methylene chloride or methanol.
   - Alkaloids, antihistamines, barbiturates and tranquilizers can be extracted into a mixture of ether and acetone. Alkaloids extract at about pH 9 and amphetamines and phenothiazines above pH 10.
   - Marijuana can be extracted from dried ground ground leaves with CH_2Cl_2.
   - Lysergic acid diethylamide (LSD) can be extracted into CH_2Cl_2 after solubilizing with a carbonate buffer;
   - Serum or urine is adjusted to pH 4 to 7.5 extract many of the drugs.
   - Extractions at pH 3 to 9 yield acidic or basic components of the drugs.
   - Barbiturates (in micro samples 0.01 to 0.05 μg) may be methylated with dimethyl sulphate before extraction into heexane
   - Drugs in powder or pill form are generally dissolved in aqueous KOH or HCl followed by extraction at appropriate pH
   - Drugs in tissues are usually protein bound. So it is necessary to precipitate protein before extraction. Macerate (grind) the tissue and treat it with sodium tungstate followed by hydrolysis with hot acid. It forms tungstic acid which is a protein precipitating agent.
2. **Methodology:** It includes the detector Flame ionization detector which is mostly used in drug screening.

   Column: OV-17 column (phenyl methyl silicone fluid)

   Other columns are:
   - PPE 20 (medium polarity)

- Carbowax 20 M (high polarity)
- Glass column with direct column injection or glass in injection port

In drug analysis, thin layer chromatography (TLC) is used to separate mixtures of medicinal agents, tinctures of medicinal plants. TLC is capable of separating drug mixtures of amino acids, alkaloids, barbiturates and sulphamylamide. TLC is specially convenient for analysing small amounts of poisonous and highly potent substances in a compound drug. The principle and other aspects of TLC are discussed below:

Principle

The drug sample under examination moves along the surface of the adsorbent. The three fold competitive interaction among solute, solvent and adsorbent establishes the relative rates at which the solvent front and the solute ascend the layer of adsorbent on the glass plate. A more polar solute is attracted to the adsorbent more strongly than a less polar solute.

Reference standards

Cocaine, heroin and methamphtamine.

Procedure

Preparation of sample solution. Dissolve 125 mg of unknown sample in 4 mL of methanol toluene (1 : 1) solution. The reference standards have been prepared at a strength of 25 mg/mL.

Application of the sample

Here commercially prepared fluorescent silica gel TLC plates supported on plastic sheets (8.5 × 3.5 cm) are used. These sheets have been activated by heating at 120ºC for 30 minutes. The sample solutions of the three reference compounds and one unknown are spotted on a single plate using fine capillary tube. The spots should be 2.5 cm from one edge of the plate and 1 cm apart. Use a fresh applicator for each sample.

Developing solvent

It consists of toluene (120 mL), acetic acid (18 mL), ether (60 mL) and methanol (1 mL). Place 5 mL of this solvent mixture in a developing chamber.

Development of the chromatogram

Dip the chromatoplate with spotted end down into the developing chamber. Since R_f values are affected by the degree of saturation of the atmosphere, so a paper impregnated with the solvent should be placed round the sides of the chamber to ensure that the air in the tank is saturated with the solvent vapour. The tank is closed firmly with the lid. When the solvent has moved to about 10 cm above the origin, the plate is removed and the solvent front is carefully marked,

Detection of the spots

The thin layer plates contain a trace of fluorescent dye, so the spots can be detected by shining an ultraviolet light on the plate. Exposure to iodine vapour often produces a colour with colourless solutes.

NOTES

Result

Calculate R_f value of the reference standards and the unknown compound. Identify the known drug component from the number, position and appearance of spots in the reference drug samples.

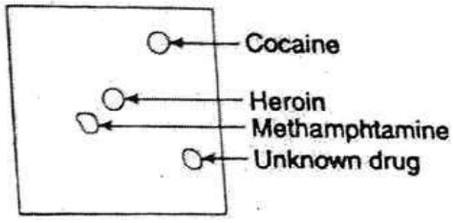
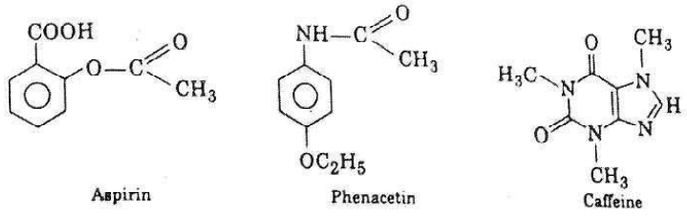


Fig. 5.15 *Thin layer chromatograms of drugs*

5.9.5 Spectrophotometric Measurements

The drug can be analysed using the following methods:

1. UV spectroscopy: A mixture of drugs such as phenacetin, caffeine and aspirin are analysed by UV method. Phenacetic exhibits UV maxima at 250 nm, caffeine at 275 nm while aspirin has maxima at 277 nm.



Reagents required

Methylene chloride, $NaHCO_3$, 4% (w/v, 1 M H_2SO_4 and HCl.)

Separation of drugs by solvent extraction

Powdered tablet is dissolved in CH_2Cl_2. Aspirin is separated from phenacetin and caffeine by extracting it into aqueous $NaHCO_3$ solution. The aqueous layer is acidified and aspirin is separated by back extraction into methylene chloride. It is measured spectrophotometrically at 277 nm. Phenacetin and caffeine, which are remaining in the original methylene chloride layer are determined in the mixture.

Preparation of standard solutions

Dissolve 100 mg/L aspirin, 20 mg/L phenacetin and 10 m/L caffeine in methylene chloride. Weigh 25 mg of each drug in a flask and make up the volume to 100 mL with methylene chloride. Since aspirin decomposes in solution, so analysis should be performed as early as possible.

Procedure

A tablet may contain 220 mg aspirin, 160 mg phenacetin and 30 mg caffeine. Grind quarter (1/4) part of the tablet to a fine powder. The method involves the following steps:

1. Add 20 mL methylene chloride into the powder with constant stirring. Transfer this mixture to a 60 mL separatory funnel.
2. Extract aspirin from methylene chloride solution with two 10 mL portions of cold 4% $NaHCO_3$ containing two drops of HCl and then with 5 mL portion of water.
3. Wash the combined aqueous extracts with three 10 mL portions of CH_2Cl_2. Add this wash solutions to the original methylene chloride solution.
4. Leave the aqueous extract in the separatory funnel.
5. Filter methylene chloride solution into a 50 mL volumetric flask and dilute to the mark with methylene chloride.
6. Again dilute 1 mL aliquot of this solution to 50 mL with methylene chloride in a volumetric flask.
7. Acidify aqueous bicarbonate solution with 6 mL of 1 M H_2SO_4 to pH 1 to 2 in the separatory funnel to prevent hydrolysis of aspirin.
8. Extract the acidified solution with eight separate 10 mL portions of CH_2Cl_2, filter into a 100 mL volumetric flask and make up the volume to 100 mL,
9. Dilute further a 5 mL portion of this solution to 25 mL with CH_2Cl_2 in a volumetric flask.

Result

Record absorbance versus wavelength curves for the standard solutions and unknown solutions between 200-300 nm.

- Using the absorbance of the standard and the unknown aspirin solution at 277 nm, calculate the percent aspirin in the tablet and milligrams of aspirin per tablet.
- Read the absorbances of phenacetin and caffeine standard's and methylene chloride extract of the sample at 250 nm and 275 nm. Using these these absorbances, calculate the percent phenacetin caffeine in the tablet and the content of each per tablet

2. Analysis of Drugs by IR spectrophotometry: IR spectroscopy can be used for the analysis of aspirin, phenacetin and caffeine (APC) in analgesic tablets. The quantitation is solely based on the intensities of the carbonyl bands.

Materials required

APC tablets, chloroform

Procedure

The drug contents of an appropriate number of tablets are directly extracted into chloroform, filtered if necessary so as to remove the insoluble tablet components. The final concentration of chloroform solution is made in such a way so that it should contain 90 mg/cm^3 of aspirin; 64 mg/cm^3 of phenacetin and 13 mg/cm^3 of caffeine. The IR spectrum is recorded in 0.1 mm NaCl-cell between 1400-2000 cm^{-1}.

Result

The intensities of carbonyl bands are observered at 1764, 1511 and 1665 cm^{-1} aspirin, phenacetin and caffeine respectively.

Check Your Progress

27. What is a drug?
28. What is a CNS drug?
29. In drug analysis, which method is used to separate mixtures of medicinal agents, tinctures of medicinal plants?

5.10 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Soil is defined as thin layer of earth crust which serve as a natural medium for growth of plants.
2. Rocks are the chief parent material from which soil are developed.
3. The approximate percentage of water in soil is 30%.
4. Water molecules held tightly on the surface of soil particles is called hygroscopic water.
5. All combustible substances with contain carbon as the main constituent are called fuels.
6. Calorific value is most important characteristic of the fuel. It may be defined as the total quantity of heat liberated by the combustion of a unit mass of the fuel in air or oxygen.
7. Boy's gas calorimeter is used for determining the calorific values of gaseous fuels or those liquid fuels which are easily vaporized
8. Flash point is defined as the minimum temperature at which the combustible liquid gives off sufficient vapours to ignite momentarily when a flame of standard dimension is brought near the surface of liquid for a prescribed rate in an apparatus of specified dimensions.
9. Blood is a red coloured fluid connective tissue, which circulates in our body.
10. Blood helps in transportation of various necessary substances such as nutrients and oxygen to the various parts of the body. It provides a means of communication between the cells of different parts of the body and external environment.
11. Plasma is the transparent fluid part of the blood.
12. Serum is the fluid obtained from clotted blood.
13. The cellular components of blood are:
    - Erythrocytes or RBC
    - Leucocytes or WBC
    - Thrombocytes or platelets
14. Clinical analysis refers to all the tests conducted in a clinical laboratory for making a medical diagnosis.
15. The serum electrolyte test includes a group of tests to measure the electrolytes such as Sodium (Na+), Potassium (K+) and Chloride (Cl-).

NOTES

16. Blood Urea Nitrogen (BUN) is a medical test that measures the amount of urea nitrogen found in blood.
17. We can obtain information regarding disease states in different organ systems by measuring the concentration of serum proteins.
18. The radioimmunoassay technique is based on the specificity of the antigen – antibody reaction.
19. In the RIA technique, at equilibrium, the amount of radiolabelled Ag* bound to antibody will decrease as the amount of unlabelled Ag will increase.
20. RIA is used in the measurement of peptide/steroid hormones, such as T3, T4, TSH, hCG, progesterone, etc., in clinical chemistry.
21. Blood gas analysis or most commonly referred as arterial blood gas (ABC1) in an essential part of diagonising and managing a patient's oxygenation status and acid base balance.
22. Normal value of venous blood pH lies between 7.32 and 7.43 and that of arterial blood between 7.35 and 7.45.
23. The pH measurement is a ratio of acids to bases and it indicates the chemical balance in the body.
24. The elements which are required in very small amounts are called trace elements.
25. Essential trace elements include iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium.
26. Hyponatremia is a condition in which the serum sodium level decreases below the normal.
27. A drug is any chemical substance that causes a change in an organism's physiology or psychology when consumed.
28. Central Nervous System Drugs CNS drugs are used to treat neurological and psychiatric problems.
29. In drug analysis, this layer chromatography (TLC) is used to separate mixtures of medicinal agents, tinctures of medicinal plants.

5.11 SUMMARY

- Soil widely vary in characteristics and properties.
- In soil water is not only important as solvent and transporting agent but it also maintain soil texture arrangements of soil particles and make it suitable for all living organisms.
- All combustible substances with contain carbon as the main constituent are called fuels.
- The fuel should have high calorific value, i.e., it should produce a large amount of heat on burning.
- Calorific value is most important characteristic of the fuel. It may be defined as the total quantity of heat liberated by the combustion of a unit mass of the fuel in air or oxygen.

NOTES

- Boy's gas calorimeter consists of a suitable gas burner in which a known volume of gas can be burnt at a known pressure and at a uniform rate (3-4 litres per minute).
- Solid fuels may be classified as natural or artificial.
- Coal, the most important solid fuel, is a mixture of various hydrocarbons formed by the combined action of high temperature and high pressure on vegetable matter over a geological period of time.
- Flash point is defined as the minimum temperature at which the combustible liquid gives off sufficient vapours to ignite momentarily when a flame of standard dimension is brought near the surface of liquid for a prescribed rate in an apparatus of specified dimensions.
- Aniline point (or standard aniline point) is the lowest temperature at which the oil is completely miscible equal volume of freshly distilled aniline.
- The carbon residue of a lubricating oil is expressed in terms of percentage of carbon that is left on evaporating a known quantity of oil under specified test conditions in a specified apparatus.
- Water gas is a mixture of carbon monoxide and hydrogen along with a small amount of carbon dioxide.
- Sample collection begins with the identification of the correct patient. Incorrect patient identification is one of the major and most dangerous sources of preanalytical errors.
- Blood is fluid mesenchymal tissue with its chief components
  - o Plasma
  - o Red Blood Cell (Erythrocytes)
  - o White Blood Cells (Leucocytes)
  - o Platelets
- The liquid part of blood consists of 55 per cent of blood volume called plasma.
- If the concentration of glucose in the blood is too high, insulin is secreted by the pancreas. Insulin stimulates the transfer of glucose into the cells, especially in the liver and muscles, although other organs are also able to metabolize glucose.
- In the liver and muscles, most of the glucose is changed into glycogen by the process of glycogenesis (anabolism). Glycogen is stored in the liver and muscles until needed at some later time when glucose levels are low.
- If blood glucose levels are low, then eqinephrine and glucogon hormones are secreted to stimulate the conversion of glycogen to glucose. This process is called glycogenolysis (catabolism).
- Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$
- Albumin is a family of globular proteins, the most common of which are the serum albumins.

NOTES

- Albumin is synthesized in the liver.
- The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins.
- A barbiturate is a drug that acts as a central nervous system depressant.
- Ultrashort-acting barbiturates are commonly used for anesthesia because their extremely short duration of action allows for greater control.
- RIA is an important technique in clinical biochemistry for estimation of hormones, steroids and drugs which are present in very minute quantities and cannot be detected by the colorimetric or other reagent methods.
- Blood gas analysis or most commonly referred as arterial blood gas (ABC1) is an essential part of diagnosing and managing a patient's oxygenation status and acid base balance.
- Blood gas analysis is performed on blood from an artery.
- Oxygen content is the actual amount of oxygen in the blood. Blood may contain less quantity that it is capable of carrying.
- Normal value of venous blood pH lies between 7.32 and 7.43 and that of arterial blood between 7.35 and 7.45.
- The elements which are required in very small amounts are called trace elements.
- These include copper, zinc, cabalt, manganese, molybdenum, iodine and fluorine.
- Calcium and phosphorus are the two most important non-protein body building materials.
- Sodium is the main cation in the extracellular fluid. About 50% of body sodium is present in the bones, 40% in the extracellular fluid and remaining 10% in the soft tissues.
- In pharmacology, a drug is a chemical substance, generally of known structure, which when administered to a living organism, produces a biological effect.
- Narcotics is a drug that produces analgesia (pain relief) narcosis (state of stupor or sleep) and addiction (physical dependence of on the drug).
- Dangerous drugs exert adverse side effects on the various body organs of the addict.

5.12 KEY TERMS

- **Soil:** Soil is defined as thin layer of earth crust which serve as a natural medium for growth of plants.
- **Calorific Value:** Calorific value is most important characteristic of the fuel. It may be defined as the total quantity of heat liberated by the combustion of a unit mass of the fuel in air or oxygen.
- **Gross Calorific Value (GCV**): It is the total amount of heat generated when a unit mass of fuel is completely burnt and the products of combustion an cooled down to 60°F, 288°K or 15°C.

NOTES

- **Net Calorific Value (NCV):** It is the net heat produced when one unit mass of fuel is completely burnt and the products are allowed to escape.
- **Flash Point:** Flash point is defined as the minimum temperature at which the combustible liquid gives off sufficient vapours to ignite momentarily when a flame of standard dimension is brought near the surface of liquid for a prescribed rate in an apparatus of specified dimensions.
- **Aniline Point**: Aniline point (or standard aniline point) is the lowest temperature at which the oil is completely miscible equal volume of freshly distilled aniline.
- **Octane Number:** Octane number of a gasoline is defined as the percentage of isooctane present in a mixture of iso-octane and n-heptane which matches the fuel (gasoline) in knocking.
- **Water Gas:** Water gas is a mixture of carbon monoxide and hydrogen along with a small amount of carbon dioxide.
- **Producer Gas:** Producer gas is a mixture of carbon monoxide and nitrogen.
- **Haemoglobin**: Haemoglobin is a metaloprotein-complex composed of haem and globin.
- **Clinical Analysis:** Clinical analysis refers to all the tests conducted in a clinical laboratory for making a medical diagnosis.
- **Electrolytes:** Electrolytes are minerals which are present in the blood and body tissues.
- **RIA:** Short for radioimmunoassay, it is an important technique in clinical biochemistry for estimation of hormones, steroids and drugs which are present in very minute quantities and cannot be detected by the colorimetric or other reagent methods.
- **Trace Elements**: The elements which are required in very small amounts are called trace elements.
- **Hyponatremia:** Hyponatremia is a condition in which the serum sodium level decreases below the normal.
- **Drug**: A drug is any chemical substance that causes a change in an organism's physiology or psychology when consumed.
- **Sedatives**: Sedatives are CNS depressant that are capable of reducing nervous tension and promote relaxation without producing sleep.
- **Tranquilizers**: These are the drugs which are used in the treatment of mental disorders.

5.13 SELF-ASSESMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. What are soil forming materials?
2. Which physical properties of soil are important for growing crops?

3. Name some inorganic compounds present in soil.
4. What are the characteristic of a good fuel?
5. Write the classification of fuel.
6. Define calorific value. What are its types?
7. How will you determine the calorific value of a solid fuel?
8. What are the various proteins found in plasma?
9. Give the structure of neutrophil.
10. What are serum electrolytes?
11. State the advantages and disadvantages of radio immuno assay.
12. Which proteins are present in blood?

Long-Answer Questions

1. Describe various types of soil found in India.
2. Analyze the composition of soil in detail
3. Explain the determination of calorific value by Boy's gas calorimeter.
4. Describe the significance of flash point determination.
5. Explain the composition and properties of water gas.
6. Elaborate the influence of pH on phosphate activity.
7. Describe the advantages and disadvantages of radioimmunoassay.
8. Analyze the process of determination of partial pressure of oxygen (PO_2) of blood.
9. Explain the trace elements in human body.
10. Explain the process of screening of drugs by gas chromatography.

5.14 FURTHER READING

Dash, Dhruba Charan. 2011. *Analytical Chemistry*. New Delhi: Prentice Hall India Pvt, Limited

Hibbert, D.B. and J.J. Gooding. 2006. *Data Analysis for Chemistry*. UK: Oxford University Press.

Benwart, G. J. 1987. *Basic Food Microbiology*. New Delhi: CBS Publishers & Distributors.

Christian, Gary D. 2007. *Analytical Chemistry*, 6th Edition. Noida: Wiley India Pvt. Limited