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BASIC ANALYTICAL CHEMISTRY

CHO-1111

Self Learning Material

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Directorate of Distance & Online Education

MANGALAYATAN UNIVERSITY
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UTTAR PRADESH

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PREFACE

In this course, we shall deal with various aspects of Basic Analytical Chemistry

- INTRODUCTION
- ANALYSIS OF WATER
- ANALYSIS OF FOOD PRODUCTS
- ANALYSIS OF COSMETICS

SYLLABUS

BASIC ANALYTICAL CHEMISTRY

UNIT 1

Introduction : Introduction to Analytical Chemistry and its interdisciplinary nature. Concept of sampling. Importance of accuracy, precision and sources of error in analytical measurements. Presentation of experimental data and results, from the point of view of significant figures. Analysis of soil: Composition of soil, Concept of pH and pH measurement, Complexometric titrations, Chelation, Chelating agents, use of indicators.

(a) Determination of pH of soil samples.

(b) Estimation of Calcium and Magnesium ions as Calcium carbonate by complexometric titration.

UNIT 2

Analysis of water: Definition of pure water, sources responsible for contaminating water, water sampling methods, water purification methods.

(a) Determination of pH, acidity and alkalinity of a water sample.

(b) Determination of dissolved oxygen (DO) of a water sample.

Analysis of food products: Nutritional value of foods, idea about food processing and food preservations and adulteration.

(a) Identification of adulterants in some common food items like coffee powder, asafoetida, chilli powder, turmeric powder, coriander powder and pulses, etc.

(b) Analysis of preservatives and colouring matter.

UNIT 3

Chromatography: Definition, general introduction on principles of chromatography, paper chromatography, TLC etc. a. Paper chromatographic separation of mixture of metal ion (Fe^{3+} and Al^{3+}). b. To compare paint samples by TLC method. Ion-exchange: Column, ion-exchange chromatography etc. Determination of ion exchange capacity of anion / cation exchange resin (using batch procedure if use of column is not feasible).

UNIT 4

Analysis of cosmetics : Major and minor constituents and their function

Analysis of deodorants and antiperspirants, Al, Zn, boric acid, chloride, sulphate. b. Determination of constituents of talcum powder: Magnesium oxide, Calcium oxide, Zinc oxide and Calcium carbonate by complexometric titration.

Suggested Applications (Any one):

(a) To study the use of phenolphthalein in trap cases.

(b) To analyze arson accelerants.

(c) To carry out analysis of gasoline.

CONTENTS

CHAPTER 1

INTRODUCTION

ANALYTICAL CHEMISTRY : ROLE

Analytical chemistry is concerned with the chemical characterisation of matter and the answer to two important questions: What is it (qualitative) and how much is it (quantitative) ? Chemicals make up everything we use or consume, and knowledge of the chemical composition of many substances is important in our daily life. Analytical chemistry plays an important role in nearly all aspects of chemistry, for example, clinical, agricultural, environmental, forensic, manufacturing, metallurgical, and pharmaceutical concerns. The nitrogen content of a fertilizer determines its value. Food must be analysed for contaminants (*e.g.*, pesticide residues) and for essential nutrients (*e.g.*, vitamin contents). The air in the cities must be analyzed for carbon monooxide. Blood glucose must be monitored for diabetes and, in fact, most diseases are diagnosed by chemical analysis. The quality of manufactured products often depends upon proper chemical proportions, and measurement of the constituents is a necessary part of quality control. The carbon content of steel will determine its quality. The purity of drugs will determine their efficacy.

The above description of analytical chemistry provides an overview of the discipline of analytical chemistry.

Analytical chemistry provides the methods and tools needed for insight into our material world, for answering our basic questions about a material sample:

- What ?
- Where ?
- How much ?
- What arrangement, structure or form ?

Analytical chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances which may be present in a material and to determine the exact amounts of the identified substances.

An Analytical chemist tries to serve the needs of many fields:

- In medicine, analylical chemistry is the basis of clinical laboratory tests which help physicians to diagnose diseases and chart progress in recovery.
- In industry, analytical chemistry provides the means of testing raw materials and for assuring the quality of finished product whose chemical composition is critical. Many house-hold products, fuels, paints, pharmaceuticals, etc. are analyzed by the procedures developed by the analytical chemists before being sold to the consumer.

- Environmental quality is often evaluated by testing for suspected contaminants using the techniques of analytical chemistry.
- The nutritional value of food is determined by chemical analysis for major components such as proteins and carbohydrates and trace components such as vitamins and minerals. Indeed, even the calories in food are often calculated from its chemical analysis.
- An analytical chemist also makes important contribution to fields as diverse as forensics, archaeology and space science.

Analytical chemistry consists of two types of analysis:

1. Qualitative Analysis
2. Quantitative Analysis

Qualitative analysis deals with the identification of elements, ions or compounds present in a sample.

Quantitative analysis deals with the determination of how much of one or more constituents is present in the sample which may be solid, liquid, gas or a mixture.

TYPES OF ANALYSIS

To select an appropriate method of analysis, following important factors must be taken into account:

(a) The nature of the information which is sought.

(b) The size of the sample available and the proportions of the constituents to be determined and,

(c) The purpose for which analytical data are required.

With respect to the information which is furnished, different types of chemical analysis may be classified as follows:

1. Proximate Analysis: In this analysis the amount of each element in a sample is determined with no concern as to the actual compounds present.

2. Partial Analysis: It deals with the determination of selected constituents in the sample.

3. Trace constituents Analysis: It is a specialised instance of partial analysis which is concerned with the determination of specified components present in very minute quantity.

4. Complete Analysis: In this analysis, the proportion of each component of the sample is determined.

On the basis of sample size, the analytical methods are again classified into following classes:

1. Macro Analysis: Macro analysis is concerned with the quantities of 0.1 g or more.

2. Meso Analysis (semimicro): This analysis measures quantities ranging from 10^{-2} g to 10^{-1} g.

3. Micro Analysis: This type of analysis deals with the quantities ranging from 10^{-3} to 10^{-2} g of sample.

4. Submicro Analysis: In submicro analysis the sample quantity ranges from 10^{-4} to 10^{-3} g.

5. Ultra micro Analysis: It deals with quantities below 10^{-4} g.

On the basis of the concentration of constituents in the sample analysis is again classified into three categories:

1. Major Constituent Analysis: It is one, which accounts for 1–100 percent of sample under investigation.

2. Minor Constituent Analysis: In this type of analysis, the minor constituent is present in the range of 0.01–1 percent.

3. Trace Constituent Analysis: Here trace constituent is analysed which is present at a concentration of less than 0.01 percent.

With the development of increasingly sophisticated analytical techniques it has become possible to determine substances present in quantities much lower than 0.01 percent upper level, set for trace constituents. Therefore a further division is made:

1. Trace Analysis: Corresponds to 10^{2}–10^{4} µg per gram, or 10^{2}–10^{4} ppm.

2. Micro trace Analysis: Corresponds to 10^{2} - 10^{-1} pg per gram, or 10^{-4}–10^{-7} ppm.

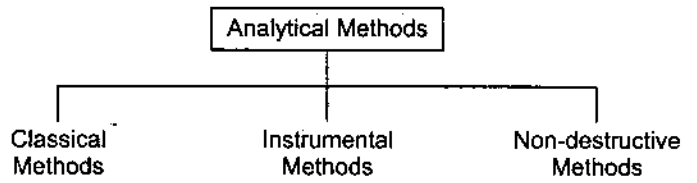
3. Nanotrace Analysis: Corresponds to 10^{2}– 10^{-1} fm per gram, or 10^{-7} to 10^{-10} ppm.

4. Subtrace Analysis: When the sample weight is less than 0.01 percent.

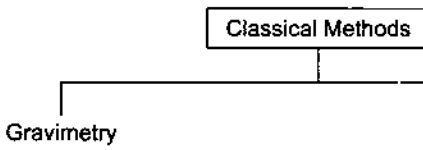
5. Ultratrace Analysis: When the sample is less than 0.001 percent.

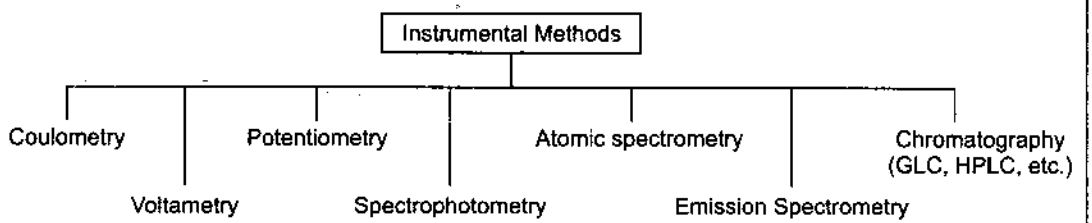
CLASSIFICATION OF ANALYTICAL METHODS

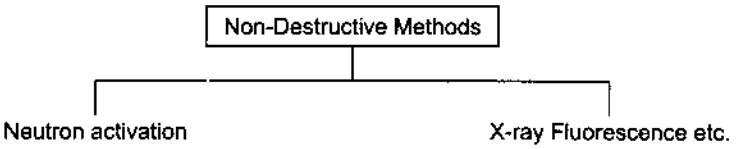
Analytical methods are divided into three categories, as follows:



The classical methods are again divided into different classes, such as:







ANALYTICAL METHODS

There are mainly three types of analytical methods available, such as:

1. Classical Methods

2. Instrumental Methods
3. Non-destructive methods.

Instrumental methods of analysis are usually much faster than purely chemical methods (classical methods). They are normally applicable at much lower concentrations than classical methods. Instrumental methods have wide applications in industry. In most cases a microcomputer can be interfaced to the instrument so that all the curves can be plotted automatically and whole analytical process may be completely automated.

SELECTING AN ANALYTICAL METHOD: Difference between Classical and Instrumental Methods

Despite the advantages possessed by instumental methods in many directions, their widespread adoption has not rendered the purely chemical or 'classical' methods obsolete. The comparison can be made by following main factors:

	Classical Methods	Instrumental Methods
1.	The apparatus required for classical methods is cheap and readily available in all laboratories.	Many instruments,which are used in instrumental methods are expensive and their use will only be justified if numerous samples have to be analysed, or when dealing with the determination of substances present in minute quantities (trace, subtrace, ultratrace analysis.)
2.	In classical methods, there is no need to calibrate a sample of known composition.	With instrumental method, it is necessary to carry out a calibration operation using a sample of material of known composition as reference substance.
3.	The classical method is suitable for a non-routine and an occasional analysis. It is often simpler to use a classical method than taking the trouble of perparing requisite standards and carrying out the calibration of an instrument.	An instrumental method is ideally suited to the performance of a large number of routine determinations.

It is clear, on the basis of above comparison that classical and intrumental methods must be regarded as supplementing each other.

FACTORS AFFECTING THE CHOICE AND SELECTION OF ANALYTICAL METHOD

It is an important task for the analyst to select the best procedure for a given determination. This will require careful consideration of the following factors:

(a) The type of analysis required: Elemental or molecular, routine or occasional.

(b) Problems arising from the nature of the material to be investigated *e.g.*, radioactive substances, corrosive substances, substances affected by water.

(c) The concentration range which needs to be investigated.

(d) The accuracy required.

(e) The facilities available.

(f) The time required to complete the analysis, this will be particularly relevant when the analytical results are required quickly for the control of a

manufacturing process. This may mean that accuracy has to be a secondary rather than a primary consideration, or it may require the use of expensive instrumentation.

(g) The number of analyses of similar type which have to be performed.

(h) Nature of the specimen.

(i) Magnitude of the sample available.

(j) Kind of information sought.

Conspectus of Some Common Quantitative Analytical Methods

Method	Speed	Relative cost	Accuracy	Concentration Range (pc)
Gravimetry	Slow	Low	High	1–2
Titrimetry	Moderate	Low	High	1–4
Coulometry	Slow-Moderate	Low-Moderate	High	1–4
Voltametry	Moderate	Moderate	Moderate	3–10
Potentiometry	Moderate-Fast	Low-Moderate	Moderate	1–7
Spectrophotometry	Moderate-Fast	Low-Moderate	Moderate	3–6
Atomic spectrometry	Fast	Moderate-High	Moderate	3–9
Emission spectrometry	Fast	High	Moderate	5–9
Chromatography	Fast	Modetrate-high	Moderate	3–9
Neutron activation	Slow	High	Moderate	–
x-ray fluorescence	Fast	High	High	–

CLEANLINESS AND NEATNESS IN ANALYTICAL LABORATORY

For an analyst it is very important to be aware of the procedures, handling of instruments and various pieces of apparatus, and basic techniques of analytical operations. The habit of clean, orderly working must also be cultivated. The following points will be helpful during handling the work in a laboratory:

1. The bench must be kept clean and a bench-cloth must be available so that any spillage of solid, or liquid chemicals can be removed immediately.

2. All glassware must be clean, and if it has been standing idle for some length of time, it must be rinsed with distilled water or de-ionised water before use. The outside of vessels may be dried with lint free glass-cloth which should be reserved exclusively for this purpose, and which should be frequently laundered, but the cloth should not be used on the inside of the vessels.

3. The working surface of the bench should not be cluttered with apparatus. All the apparatus associated with some particular operation should be grouped together on the bench, this is most essential to avoid

confusion when duplicate analysis or determinations are in progress. The apparatus which are not in use, should be immediately returned to the locker.

4. The container vessels or bottles must be labelled, so that the contents can be readily identified.

5. To prevent contamination of the contents by dust, air and moisture, the vessels should be covered immediately after use.

6. Bark corks should not be used to cover the vessels because they invariably tend to shed some dust.

7. For Temporary labelling, a 'chinagraph' pencil or a felt tip pen (Glass marker pen) should be used, not the gummed labels, which are used when more permanent labelling is required.

8. Reagent bottles must never be allowed to accumulate on the bench, they must be placed on the reagent shelves immediately after use.

9. All determinations should be perfomed in duplicate.

10. A stiff covered notebook of A4 size must be provided for recording experimental observations as they are made. A double page should be used for each determination, the title of which, together with the date, must be clearly indicated. One of the two pages must be reserved for the experimental observations, and the other should be used for a brief description of the procedure followed.

11. The record must conclude with the calculation of the result of the analysis and in this connection the equations for the principal chemical reactions involved in the determination should be shown together with a clear exposition of the procedure used for calculating the result. Finally, appropriate comments should be made upon the degree of accuracy and the precision achieved.

12. The graph paper or 'printout' obtained from a printer of modern instrument, should be attached to the observation page of the laboratory record book.

13. It should be regarded as normal practice to perform a rough calculation to confirm the right order of printed results.

14. Safety procedures must be observed in the laboratory at all times.

15. Poisonous chemicals must be handled very carefully.

16. All laboratory workers should familiarise themselves with local safety requirements.

17. In some laboratories, the wearing of safety spectacles and gloves must be compulsory.

LABORATORY NOTE BOOK

In the laboratory, all data should be recorded permanently in ink, when they are collected. This orderliness will give extra advantages. First of all there will be saving of time in not having to recognize and rewrite the data, and chances for a mistake will be reduced. Second advantage in an immediate record is to be able to detect possible errors in the measurements or calculations. And data will not be lost or transferred incorrectly, if they are recorded directly in a notebook instead of collecting on scraps of paper.

The laboratory note book is a record of the work of an analytical chemist. It is the source for reports, publications and regulatory submissions. The note

book is a record of original ideas. The success or failure of a company's product or service may depend on how well it is documented.

Some good rules are given below for a well-maintained note book:

1. A hardcovered notebook of A4 size must be used for recording experimental obserations as they are made.
2. Never use loose leafs.
3. Number the pages consecutively.
4. Always record only in ink.
5. Never tear out pages. If page is not used, put a line through the page.
6. Each page should be dated and signed properly.
7. Name of the project should be written on the proper space.
8. All the data should be recorded on the same day, when they are obtained.
9. In a note book, a double page should be devoted to each determination, title of which must be clearly indicated. One of the two pages must be reserved for the experimental observations, and the other should be used for a brief description of the procedure followed, but with a full account of any special features associated with the determination.
10. In most cases it will be found convenient to divide the pages on which the experimental observations are to be recorded into two halves by a vertical line, and then to half the right hand column thus created by a second vertical line. The left-hand side of the page may then be used to indicate the observations to be made, and the data for duplicate determinations can be recorded side by side in the two right-hand columns.
11. The record must conclude with the calculation of the result of the analysis and in this connection the equations and reactions involved in the determination should be shown together.
12. Finally, appropriate comments should be made upon the degree of the accuracy and the precision achieved.
13. Many modern instruments used in the analytical laboratory are interfaced with a computer and a printer provides a permanent record of the experimental data and the final result may even be given. The printout should be permanently attached to the observations page of the laboratory record book.
14. It should be regarded as a normal practice to perform a 'rough' calculation to confirm that the printed result is of the right order.

SAFETY IN THE ANALYTICAL LABORATORY

Before beginning any experiment, one should be familiar with laboratory safety procedures. Good housekeeping practices will ensure the safest working conditions in the laboratory. The general safety rules in the analytical laboratory are given below:

1. In the laboratory, always clean up spilt chemicals.
2. Broken or chipped glassware should not be left on the bench or shelves.
3. Chemical bottles and apparatus should be placed properly, after use.
4. One should neutralise the acid spills with sodium bicarbonate and alkali spills with boric acid.

5. Mercury spills should be vacuumed up with a suction flask or dusted with sulphur powder.

6. Analyst should clear up the mercury thoroughly because mercury vapours from fine droplets are highly toxic.

7. If required, one should wear protective glasses, while working in the laboratory.

8. A person working in a chemical laboratory should locate fire extinguishers, exits, safety showers, eye fountains, and fire blankets.

9. Any dangerous or potentially dangerous laboratory situation should be brought immediately to the notice of the laboratory supervisor.

10. One should perform only the authorized experiments, and should not work alone in the laboratory.

11. When working with volatile chemicals, as when heating acids or when using organic solvents, use the fume hood.

12. One should use a safety shield, when working with potentially dangerous reactants.

13. Special care should be taken when working with organic solvents.

14. Many chemicals are inflammable and many have been identified as acute or chronic toxic substances, frequently carcinogenic. Use rubber gloves when possible and avoid breathing in fumes.

LABORATORY OPERATIONS AND PRACTICES

For a quantitative analysis there are many laboratory operations and techniques. These are described as follows :

(1) Filtration (2) Drying (3) Measuring volume (4) Stoichiometry and Graphs (5) Concentration (6) Percentage solute (7) Activity (8) Titration of a solution (9) ppt and ppm (10) Standard (11) Chemical standard (12) Sampling (13) Drying (14) Weighing (15) Dissolving (16) Dcomposition of organic matter (17) Ionisation (18) Precipitation

1. FILTRATION

Filtration is the separating process of solid phase from the liquid phase which is present in its contact solution. The filtering process uses different types of filter-support to collect the solid, such as (A) filter papers, (B) crucibles, (C) filter pulp mats, (D) asbestos, (E) filter membranes etc.

(A) Filter paper: Filter papers are available commercially in different pore sizes. The filter paper is chosen according to the size of the particles of the solution being filtered. The most common and the best quality of filter paper is Whatman filter paper. Whatman filter paper is found in 3 different grades such as:

(a) Quantitative grade, (b) Semi-quantitative grade, (c) Qualitative grade.

The Whatman filter paper is an ashless paper which, on combustion, leaves no ash or paper residue to be detected by the balance (Fig.1).

(a) Quantitative Grade: Whatman No. 40 filter paper is generally used for filteration of quantitative work. This filter paper is made of pure cellulose fibres. It is also washed with HCl and HF until it is free from silica and other inorganic sources of ash. Whatman No. 41 filter paper is used to filter the gelatinous precipitates such as iron hydroxide, aluminium hydroxide or silica acid. Whatman No. 42 filter paper is a close texture grade which is used for

finely divided precipitates. Whatman No. 50 filter paper is acid hardened and it is used specially with vacuum.

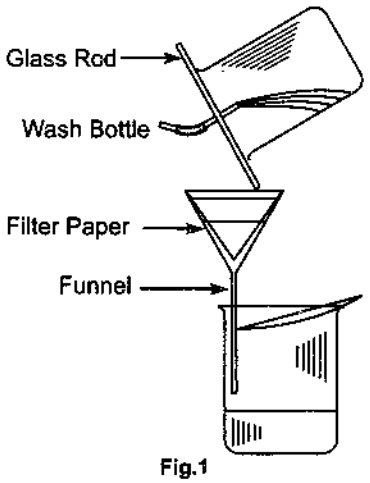


Fig.1

(b) Semi-Quantitative Grade : For semi-quantitative work Whatman No. 30 filter paper is most commonly used. It is known for rapid and fine filtration. It is also acid washed Filter paper. Whatman No. 31 filter paper is used to seperate the gelatinous precipitates from the solution. Whatman No. 32 filter paper is used for the filtration of fine precipitates. Semi quantitative fitter paper is also an ashless paper, the ash of an 11 cm circle of these papers is about 0.00003 gram in weight.

(c) Qualitative Grade: This grade of filter paper is HCl washed and it is an ashless filter paper. Whatman No. 1 paper is known as qualitative grade filter paper which is used to filter the precipitates of average fineness. Whatman No. 2 paper is thicker than Whatman No. 1 and hence it is not used for fast and rapid filtration. Whatman No. 3 paper is known to filter the very fine precipitates although it is a thick filter paper. Whatman No. 4 filter paper helps to filter the gelatinous precipitates rapidly. Whatman No. 5 filter paper is used with a vacuum, it is hard, tough and of close texture. All these filter papers are used when precipitate is only to be preserved. When precipitate has to be weighed and ignited, the qualitative grade filter papers are not used.

Other Types of Filter Papers

There are some other types of filter papers available commercially such as:

(i) Munktels (Swedish filter paper)

(ii) Carl Schiecher (German paper)

(iii) Schull (German paper)

(B) Crucibles: Filterpaper cannot always be used for filtration. For example, the precipitate might slowly react with the cellulose or the precipitate might not be stable at the temperature required for charring the filter paper. In these cases, filltration of a precipitate is done under vacuum in crucibles. There are following types of filtering crucibles:

(i) Porous Glass fritted disc crucibles: These crucibles are also known as sintered glass crucibles. Depending upon the pore size these crucibles are graded as G–1, G–2, G–3, and G–4. These are made of excellent quality of

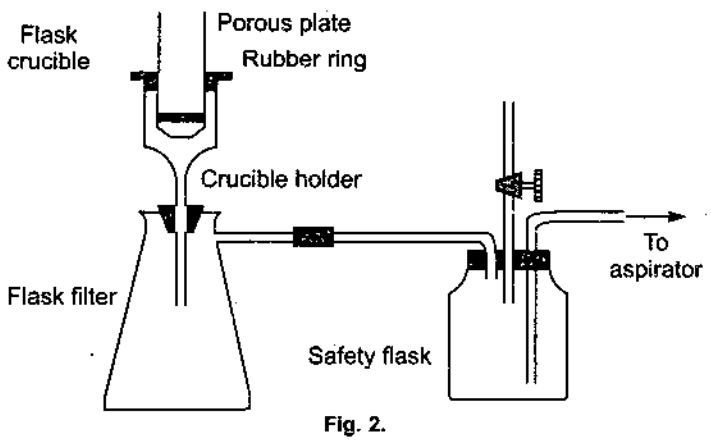


Fig. 2.

resistance pyrex or jena glass, and have transparent glass sides and a porous fritted glass bottom. In these crucibles a sintered ground glass filter disc is fused at the bottom of the crucible. These crucibles can be brought to constant weight easily (Fig. 2.)

(ii) Gooch Crucibles: The precipitates, which are quickly reduced by the carbon of the filter paper on ignition, are preferably filtered by Gooch crucibles. The disc of these crucibles consists of large holes. These holes are covered by an asbestos mat or other filtering materials.

In place of vacuum, these crucibles contain a thin layer of acid and alkali washed asbestos as a filtering medium. It is more difficult to get a constant weight of a Gooch Crucible. These are fitted to either glass funnel with rubber rings or to a Gooch crucible holder.

(iii) Other types of crucibles

Aluminium crucibles: These are made of aluminium oxide fritted together. The main drawback of these crucibles is that they have a tendency to lose uniformity in weight on repeated combustion and ignition.

Porcelain Crucibles: For common and routine experimental work a plane porcelain crucible is used for heating the precipitate.

Silica crucible: Silica crucibles are more commonly used for heating and cooling purposes, without cracking. These crucibles are more safe.

Platinium crucibles: Platinim crucibles are the best crucibles for heating the precipitates, although these are very expensive.

(c) Filter Membranes: These are synthetic filter membranes which have been developed in recent years. These are not used in gravimetric analysis. These membranes are used in filtering devices and apparatus and are uniform micro porous screens with millions of pores per sq. cm or surface area. Filter membranes are useful in the filtration of dust from air, high molecular weight proteins and other large molecules.

(d) Filter pulp: Filter pulp is commercially available in the form of small pallets and in moist form for qualitative and quantitative analysis. The gelatinous silica, finely divided barium sulphate and colloidal hydrated oxides etc. have been filtered by using filter pulp with the precipitates.

Factors on which filtration depends

The technique of filtration depends upon the following factors:

(i) Size of the particles to be filtered.

(ii) Chemical reactivity of the particles.

(iii) Purpose of filtration

The speed of filtration of any filter paper decreases as its pores become clogged with precipitate. Thus it is advisable to allow the precipitate or insoluble matter to settle before filtration. The process of settling and filtration can be speeded up by adding a slurry of filter paper in the mother liquor or solution after precipitation.

2. DRYING

For drying purpose desiccator is used. It allows to cool the hot crucible in a dry atmosphere, because desiccator provides a dry and moisturefree atmosphere for storing a sample (Fig. 3).

A desiccator consists of a glass or aluminium vessel with a tightly fitted lid and a large space to fill the desiccant. The top of the desiccator and the cover

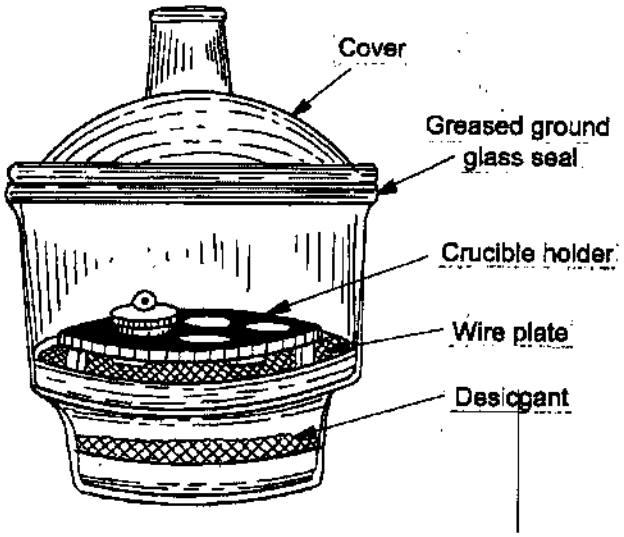


Fig. 3. A desiccator

are ground flat and a thin layer of grease or petroleum gel is applied between them which helps as an air tight seal. The lid of desiccator is opened by sliding. The desiccator is used to keep the hot crucibles to protect them and their contents-from dust, moisture and hot laboratory flames as well as to cool at room temperature.

The desiccator or dry agents are used in the desiccator, some of them are:

(1) anhydrous calcium chloride, (2) fused NaOH, (3) calcium oxide, (4) sodalime (mixture of NaOH and CaO), (5) anhydrous $CaSO_4$, (6) magnesium oxide (MgO), (7) P_2O_5, (8) silica gel, (9) conc. H_2SO_4 etc.

When placing the hot objects into the desiccator, one must be careful. When hot material is placed in the desiccator and the lid is tightly closed, the hot object of the desiccator warms the inner air, which contracts on cooling and produces a partial vacuum that makes removal of the lid very difficult. Sometimes, when the lid of desiccator is opened, air will suddenly rush into it and spill the sample by blowing it out of its container. Thus it is advisable that hot object be first allowed to cool for about 1 minute in air before putting it in the desiccator.

3. MEASURING VOLUME

In analytical chemistry solutions are measured by graduated glasswares such as volumetric flask; pipette, burette etc. Proper handling and use of glassware in volumetric analysis needs an accurate reading of the liquid or solution level. The lowest point of solution level (bottom of the meniscus) should be read as the correct volume. For the detection of meniscus in coloured solutions, a small piece of white paper or card board behind the meniscus generally helps in reading. To read a level of volume, eye should be put at parellel height of the meniscus (Fig. 4).

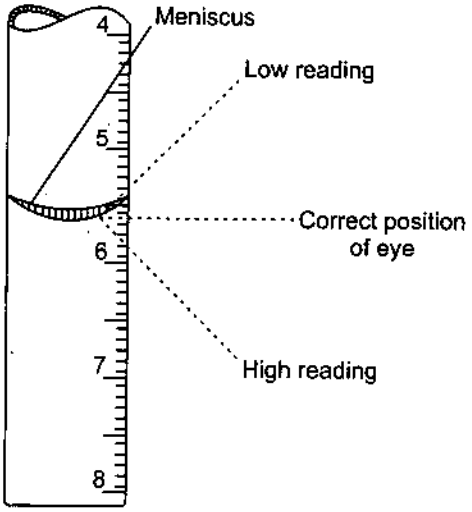
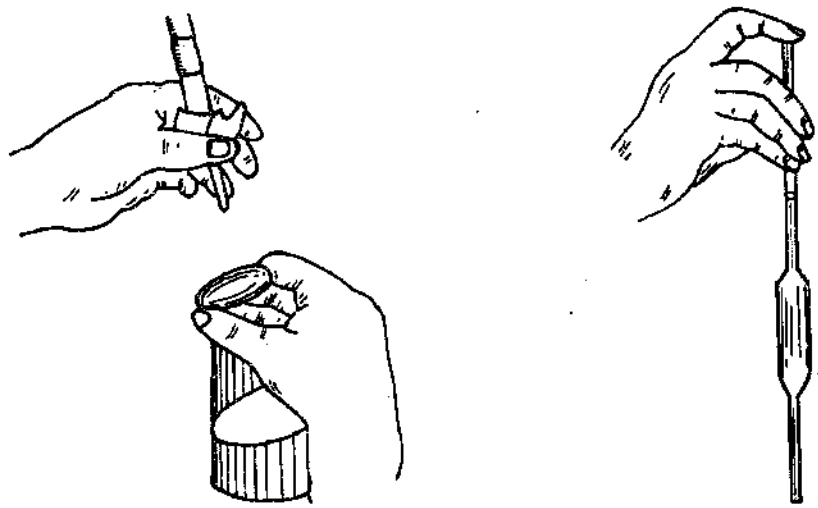


Fig. 4. Technique for reading a menicus

Pipette: Before using a pipette, it should be rinsed with a small portion of the liquid which has to be pipetted out. If the pipette is wet with water, it should be rinsed with the solution many times. First of all check the flow of the pipette. If it tends to form beads, it shows that pipette is dirty and should be cleaned. In a pipette the solution should be filled to a level of about 2–3 cm above the calibration mark. After filling the pipette its tip and stem should be wiped dry with tissue or filter paper. The pipette is now held vertically and solution is allowed to come down until the meniscus reaches the calibration mark. After complete drainage of the liquid, the tip of the pipette is touched to the wall of the vessel for about 10 second (Fig. 5).



Manipulation of stopcock of a burette.

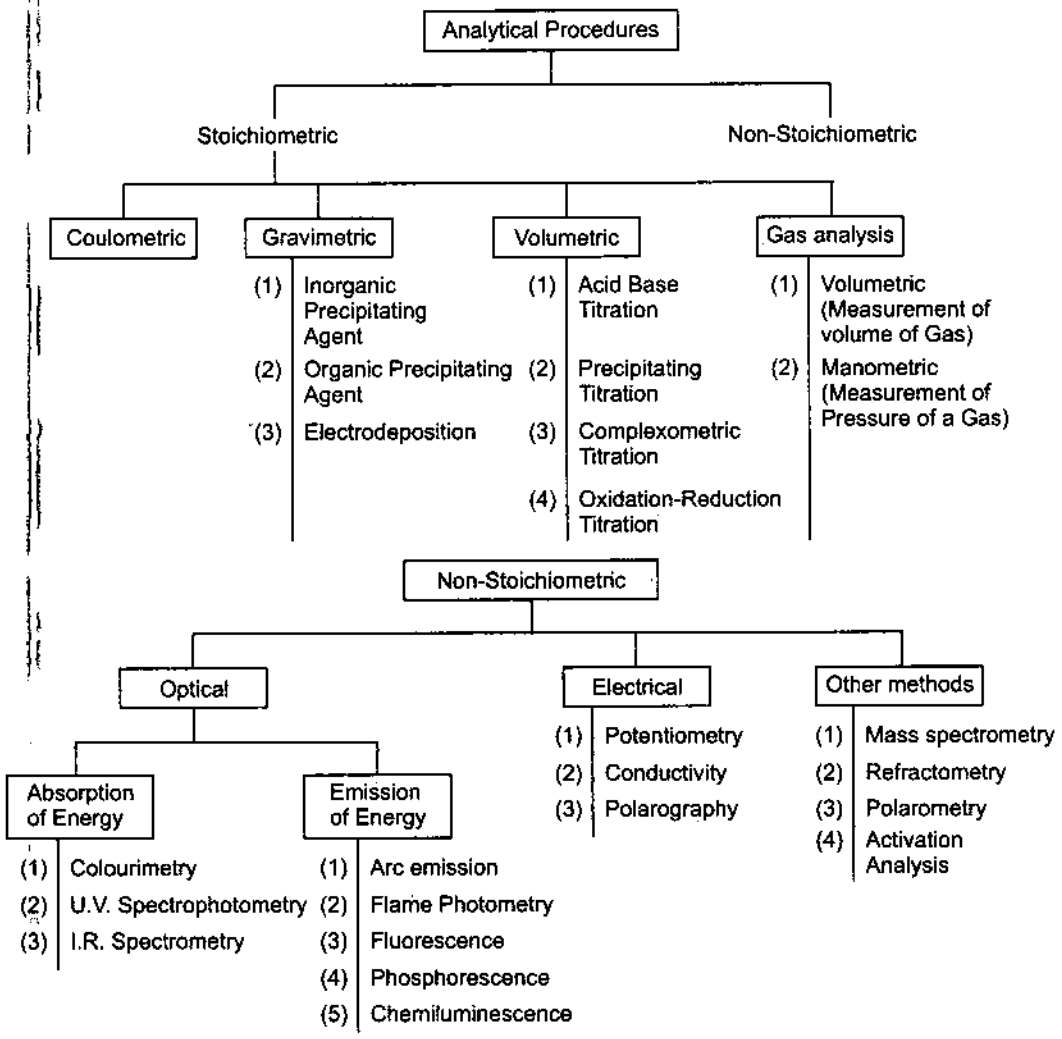
Holding of pipettle in vertical position.

Fig. 5

Volumetric Flasks: Volumetric flasks are useful to prepare the standard solutions of known concentration. The exact known weight of the solute should be taken into the volumetric flask with the help of a very short wide stemmed funnel. Now the solvent is added into the flask and the solution is swirled very carefully until the solute dissolves. At the completion of dissolving of the solute little more solvent is added upto the mark, with the last few millilitres of solvent added very carefully.

To prepare a solution of known concentration, another process can be performed. First transfer the correctly weighed sample into a large beaker and dissolve properly in the solvent. The prepared dissolved solution is then transferred into the volumetric flask and made up to the mark by adding a little more solvent. In both procedures it is necessary to dissolve the solute completely before the final dilution is made. Place the stopper into the flask, after the complete dilution. Now invert the flask back and forth at least 4–5 times by placing the thumb over the glass stopper to ensure the complete mixing of the solution.

Burette: In volumetric analysis, when titration has to be performed with the use of a burette, one hand is used to swirl the titrant container and the other is used to control the stop cock. Before using burette, it should be rinsed with the titrant solution. There should be no air bubbles or beads of titrant on the inner surface of the burette. The tip of the burette should be 2–3 cm. above the solution surface in the titration vessel.



4. STOICHIOMETRY

Analytical methods can be seen as:

The analytical procedures may be of two types:

(i) stoichiometric; (ii) non-stoichiometric

(i) Stoichiometric: In stoichiometric method the constituent whose amount has to be measured undergoes a reaction with another substance or it is allowed to decompose in accordance with a well defined equation, like

$$\text{Reactants} \longrightarrow \text{products}$$

$$R_A + R_B \longrightarrow P_C + P_D$$

where, R_A = Desired constituent, R_B = Reacting Reagent

The quantity of desired constituent R_A can be measured by applying the laws of definite and combining proportions, provided the quantity or amount of any of the products (P_C *or* P_D) or of the reagent used is known or can be calculated.

(ii) Non-Stoichiometric: Non-stoichiometric analytical procedures are just opposite to the stoichiometric. Non-stoichiometric procedure can not be written as exact well defined reactions and equations. In many cases, non-stoichiometric procedures are based on the measurement of a physical property which changes in the proportion to the concentration of the desired constituents. Since a number of physical properties may be measured with great accuracy, it is then only necessary to calibrate the procedure. The

calibration defines the relationship between the concentration of the desired constituent and the magnitude of the physical property under suitable proper conditions. In gravimetric and volumetric methods (wet chemical methods), some separation procedures are stoichiometric while most of the instrumental methods are non-stoichiometric. Some example are:

Gravimetric method, titrimetric method, gas analysis and coulometric etc. methods are stoichiometric whereas optical, electrical, mass spectrometry, refractrometry, polarimetry and activation analysis etc. are the examples of non-stoichiometric procedures.

GRAPHS

In analytical chemistry, the experimental observations are analysed and measured by calibration and titration curves.

Calibration Curve: A calibration curve is a graphical co-relationship of instrumental response to concentration. The concentration of an unknown is established by treating the unknown in the same way as the standards which were used for preparing the calibration curve. When the instrument response for the unknown has been determined its concentration can be read directly from the calibration curve.

Titration Curve: An end point of titration can be determined in a number of ways. The fastest observation is its location by inspection. In another method, the rate of change in the property is plotted on the Y-axis w.r.t. a small constant increment of titrant. The values, $\Delta Y / \Delta V$ are plotted against the volume added. This is known as differential titration curve.

5. CONCENTRATION

In stoichiometry reactions, concentration is generally defined in terms of (i) molarity (ii) normality (iii) formality. Concentration of a gas is commonly expressed by its pressure. The terms molarity, formality, and normality can be expressed in the following manner.

(a) Molarity: Molarity is denoted by M and unit of concentration. It can be defined as the number of moles of solute dissolved per litre of solution, therefore,

$$\text{Molarity (M)} = \frac{\text{Moles}}{\text{Volume of solution in litre}}$$

For example, 1M solution of KCl can be prepared by dissolving 1 mole of KCl (74.55 gm) in water or some other solvent, to get exactly 1 litre of solution. Since KCl is completely dissociated in solution, the solution will be exactly 1 M in K^+ and 1 M in Cl^- ion, if water is taken as solvent. Molar concentration in this case is zero. If KCl is partially dissociated, its molar concentration would be determined by the amount of dissociation taking place.

(b) Formality: It is denoted by F and can be expressed as the number of the gram formula weight (GFW) of the solute dissolved per litre of the solution. Thus,

$$\text{Formality (F)} = \frac{\text{Gram formula weight}}{\text{Litre of solution}}$$

For example, in case of KCl solution the concentration can be defined as 1 F KCl. The concentration can also be expressed as 1 F K^+ and 1 F Cl^- as KCl is completely dissociated.

(c) Normality: Normality is denoted by N and can be expressed as the number of gram equivalent weight of solute per litre of solution.

$$\text{Normality (N)} = \frac{\text{Gram equivalent weight}}{\text{Litre of solution}}$$

Normality varies according to the reaction in which the solute participates. Therefore, accurate calculation of gram equivalent weight can be made after determining the specific changes in identity of the solute in the course of chemical reaction.

6. PERCENTAGE SOLUTE

The percentage of solute may be defined as percentage by weight or percentage by volume. When percentage solute is expressed as percentage by weight, it means the part of the total solution weight that is participated by the solute.

For example, 2% H_2O_2 solution (by weight) shows 2 gram of H_2O_2 in 100 gram of solution or 2 gram of H_2O_2 dissolved in 98 gram of water. The weights of solute and solvent are additive.

If the above solution is prepared as 2% H_2O_2 solution (by volume), it means 2 mL of H_2O_2 is diluted to a total volume of 100 mL. It does not mean that 2 mL of H_2O_2 and 98 mL H_2O are mixed. The correct volume (% by volume) would not be obtained by mixing 2 mL and 98 mL. In fact, contraction and expansion will occur and its magnitude depends on the property and nature of solute and as well as its physical state such as solid, liquid or gas.

7. ACTIVITY

Ideal concentration like normality, molarity or formality corresponds to what was originally added and true or effective concentration which takes into account the various interactions, is called the activity.

For dilute solution, the activity is proportional to the concentration of solute

$$a \propto C$$

or

$$a = \gamma C$$

where, a = activity, C = concentration γ = proportionality constant, and known as activity co-efficient.

It is important to know that in ordinary analytical determinations and in volumetric and gravimetric procedures, molar concentration of a substance is determined rather than activity.

8. TITRE

The Titre value of a solution may be expressed as the weight of a pure substance which is chemically equivalent to react with constant volume (generally 1 mL) of the solution.

9. PARTS PER THOUSAND AND PARTS PER MILLION

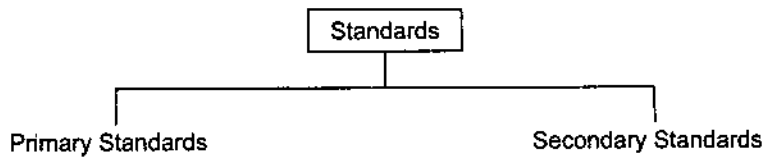
Percentage concentration of a solution on weight basis is generally defined in terms of parts per thousand (p.p.t.) or parts per million (p.p.m.). In terms of weight, the unit of p.p.t. is milligram per kilogram (mg/kg) or microgram per gram (μm/g).

For example, 1 p.p.t. Sn in an alloy indicates that the alloy consists of 1

part by weight of Sn in 1000 parts by weight of alloy. Similarly, a solution of 1 p.p.m. Sn implies 1 part Sn by weight in one million parts by weight of sample solution.

10. STANDARDS

For all types of determinations, the establishment of a standard or reference point is necessary. In chemistry, a substance whose purity has been analysed may be a primary standard substance. The primary standard (or reference point) is a precisely defined unit of measurement for determining the physical properties. Primary standard used in analysing mass, time, length and wavelength. As primary standards are not always available, other reference materials which are closely satisfied with the requirements are used. These are known as secondary standards.



Physical property	Unit	Definition of Unit
Mass	Gram	1/1000 the quantity of matter in the international prototype kilogram.
Length	Centimetre	1/100 the length of international prototype metre at 0 ⊃C or 1553164.13 times the wavelength of red Cd line in air at 760 mm. Hg at 15 ⊃C.
Time	Ephemeris second	1/31556825.9747 of the tropical year for 1900 January O^d 12^h ephemeris time 1 ephemeris day = 85,400 ephemeris seconds.
Wavelength	Ångstrom	6438–4686⊃A, which is wavelength for the red radiation from Cd relative to the metre (adapted 1907).

11. CHEMICAL STANDARDS

All analytical methods require a chemical standard. For example, in volumetric titration, measured volume of a reagent of known concentration is added to a solution of an unknown concentration or to a solution of the substance to be analysed. Addition is continued upto stoichiometric end point at which amount added is stoichiometric to the quantity of substance to be analyzed. In order to calculate the amount of unknown substance in the solution, it is important to know the concentration of the reagent solution precisely and such a solution is known as standard solution.

Standard solution: The solution of a primary chemical standard can be made directly by exactly weighing the substance with an analytical balance and then dissolving it to a known volume in a volumetric flask. In this way the prepared solution is called as standard solution of the known concentration. The concentration of this solution can be expressed in terms of molarity, molality, normality, or formality as the case may be.

A standard solution of known acidity is prepared if primary standard is

acidic. A standard solution of known basicity is obtained, if primary standard is basic.

A substance whose standard solution can not be prepard directly by weighing is known as secondary chemical standard and its solution can be prepared by standardizing against a standard solution of a primary standard.

EXAMPLE

Standard solution of sodium hydroxide (NaOH) can not be prepared directly by weighing NaOH pallets, because it is secondary standard and it does not meet the requirements of a primary chemical standard. Therefore, a solution containing the approximate concentration of NaOH is first prepared and it is then standardized against acidic primary standard solution of known concentration (standard solution). The prepared NaOH solution is titrated against standard acidic solution to the stoichiometric point and the concentration of the unknown NaOH solution is calculated from the amount of primary standard used, since reaction stoichiometry is known. The NaOH solution is now standardized and can be called as a secondary standard solution.

Essential Requirements of a Primary Chemical Standard

The essential requirements of a primary chemical standard are as follows:

(a) The cost of primary standard should be reasonable.

(b) It should be highly pure (*e.g.*, upto 99.99%).

(c) The primary standard should be stable in nature.

(d) It should be non-hygroscopic.

(e) Primary standard should undergo stoichiometric reactions.

(f) It should be completely soluble in the solvent used.

(g) The molecular weight of primary standard should be high.

12. SAMPLING

Sampling is an important operation to analyse a material or substance. It is a difficult task to get a proper and homogenous sample. A lot of time and efforts will be involved in the analysis of an improper sample. Sampling is of two types:

(a) Statistical sampling: A sample for analysis may either be selected at random or according to a thorough plan which provides every particle and portion of the substance an equal chance of appearing in the sample. This plan is known as statistical sampling. In this plan portions from every section are removed and then combined, mixed well and again divided into small sections to prepare laboratory size sample.

(b) Random sampling: Sampling by random choice is difficult, because crushing, grinding, labeling and storage of the sample are employed in it and any of these operational techniques may alter the composition and properties of the original sample.

There may be two types of sample mixtures:

(a) Heterogenous: Heterogenous mixtures such as emulsions, powders, suspensions or aerosols, which are handled by statistical plan.

(b) Homogenous: In homogenous samples, all the particles of sample are well mixed and uniformed. Handling of an homogenous sample is easy.

On the basis of physical state there are 3 types of samples:

(a) Solid, (b) Liquid, (c) Gas

(a) Solids: In the sampling of solids, the particle size is mainly considered. As the composition of particles of various size may change. Large particle samples are converted into small and suitable size for analysis, according to the followig procedure;

(i) The sample is first converted into uniform particle size.

(ii) Then mass of the sample is reduced.

The size of uniform particles is obtained by passing the original sample through crusher, mills, mortar and pulverizers and then sieving. Sampling of large mass may be carried out either by hand or by mechanical sampling machine.

(b) Liquids: With the help of any suitable sampling device the pure and homogenous liquid is taken for sample. Attention should be paid to preserve the purity and homogenity of the original liquid sample. Additional care should by taken if the liquid mixture is unstable, consists of volatile components or disolved gasses. For sampling of a liquid, samples are withdrawn from different depths and from all locations in the liquid sample. They are either analysed separately; or combined and a composite sample is obtained.

(c) Gases: The samples of gases are collected by expansion into an evacuated container, flushing and displacement of a liquid technique. Sampling devices for gases are usually made from glass and are fitted with stop cocks at both the ends. If some contamination occurs form the previous sample, it can be removed by extensive flushing of the container with the gas to be sampled.

It is very difficult to collect an atmospheric sample, because of the presence of wind, heavy pollution and rain. These problems can neither be controlled nor overcome. Usually atmospheric air samples are passed into the trapping or filtering system device at a controlled flow rate. In the filtering device the air sample is passed through a series of fine filters, here the filtering action is controlled by the porosity of the filtering device.

Sampling on Moon Surface: It is a very difficult task of sampling on moon because statistical selection of the moon surface was difficult, hence the sampling is possible there on the basis of size and physical state of limited quantity of moon rocks.

However, in all cases the aim of sampling is statistical and the use of common sense. It is not very feasible to describe a set of general procedure for sampling of all types of substances under all conditions.

13. DRYING

After getting the sample, it is important to know whether sample has to be used as such, or it has to be dried. Some samples are hygroscopic in nature or may contain moisture. Hence there are 2 types of analysis.

(a) Received Basis: If analysis of sample is done on 'received basis' (as such it is) it means water is a part of sample-composition and therefore there should be no gain or loss of water of the sample before analysis.

(b) Dried Basis: It means sample has water to be removed before analysis by heating in an electric oven, muffle furnace, Bunsen burner or by Meeker burner. There should be a very careful heating otherwise sample may

be destroyed, decomposed or may loose volatile components. The dried sample must be cooled in a desiccator and stored or preserved in absence of water.

14. WEIGHING

Samples are weighed after the drying process. Generally an analytical balance is used for this purpose. The weighing must be employed in triplicate. After weighing, the sample is dissolved in a volumetric flask to a known volume. Aliquots of this dissolved solution are used for quantitative analysis.

15. DISSOLVING

After weighing process, the sample is dissolved thoroughly. Inorganic salts are soluble in water whereas organic compounds are soluble in the organic solvents. Sometimes, inorganic samples are slowly ionised in water and form insoluble hydrous oxides. The solubility of some inorganic salts in water is given below:

S.N.	Inorganic Salts	Soluble	Partially Soluble	Insoluble
1.	All acetates except $AgC_2H_3O_2$, $Hg(C_2H_3O_2)_2$	✔	–	–
2.	Arsenates; borates and carbonates of Na^+, K^+, NH_4^+	✔	–	–
3.	Bicarbonates of Na^+, K^+, NH_4^+, Ba^{2+}, Ca^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Sr^{2+}	✔	–	–
4.	Fluorides of Na^+, K^+, NH_4^+, Ag^+, Sn^{2+}, FeF_3	–	✔	–
5.	Oxalates of Na^+, K^+, NH_4^+, Fe^{3+}	✔	–	–
6.	Chromate of Na^+, K^+, NH_4^+, Cu^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}	✔	–	–
7.	Phosphates of Na^+, K^+, NH_4^+	✔	–	–
8.	Sulphates like $BaSO_4$, $SrSO_4$, $PbSO_4$, Ag_2SO_4, $CaSO_4$	–	✔	–
9.	Sulphite like $MgSO_3$	–	✔	–
10.	Nitrite, such as $AgNO_2$	–	✔	–
11.	Sulphides	✔	–	–
12.	But Na_2S, K_2S, $(NH_4)_2S$, BaS, SrS, CaS, MgS	–	✔	–
13.	Chloride and bromide of Ag^+, Hg_2^+	–	–	✔
14.	Iodide of Ag^+, Hg_2^{2+}, Pb^{2+}	–	–	✔
15.	$PbCl_2$, $PbBr_2$ and $HgBr_2$	–	✔	–

16. DECOMPOSITION OF ORGANIC MATTER

The decomposition of organic matter can occur by various procedures but there are two common methods which have generally been used;

(i) Wet Ashing

(ii) Dry Ashing

The selection of these methods depends upon two factors:

(a) Only organic removal is required; or

(b) Whether analysis of organic matter is desired.

(i) Wet Ashing: This method of decomposition of organic matter requires HNO_3 - $HClO_4$ mixture. This mixture should be handled very carefully, otherwise it causes a violent explosion. In this method HNO_3 is added first, heated, cooled and then HNO_3 - $HClO_4$ mixture is added. Nitric

acid acts here as a moderating force by oxidising the more reactive compounds at lower temperature.

(ii) Dry Ashing: In dry ashing method the organic matter is heated in an open crucible in presence of air or oxygen to red hot until all the carbonaceous material has been oxidised. In this procedure the loss of sample by sputtering and volatilization should be avoided.

17. IONISATION

When an inorganic salt is dissolved in water, it undergoes ionisation. This salt is also known as electrolyte. It is of two types.

(a) Strong electrolyte: When dissolved in water, ionisation takes place completely such as:

$$NaCl\ (s) \xrightarrow{H_2O} Na^+ + Cl^-$$

$$NaOH\ (s) \xrightarrow{H_2O} Na^+ + OH^-$$

$$HCl\ (g) \xrightarrow{H_2O\ H^+} + Cl^-$$

(b) Weak electrolyte: When dissolved in water, weak electrolytes act as follows:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

$$HC_2H_3O_2 + H_2O \rightleftharpoons H_3O^+ + C_2H_3O_2^-$$

The reactions involved in weak electrolytes are typical equilibrium reactions and can be expressed in terms of equilibrium constants. Thus

$$K = \frac{[H_3O^+]\,[OH^-]}{[H_2O]^2}$$

$$K_a = \frac{[H_3O^+]\,[C_2H_3O_2^-]}{[HC_2H_3O_2]}$$

$$K_b = \frac{[NH_4^+]\,[OH^-]}{[NH_3] + [H_2O]}$$

Here K, K_a, K_b are called ionisation constants or dissociation constants. The magnitude of $K_a, (K_b)$ is a measure of strength of weak electrolyte as an acid (base). The larger the K_a or K_b the stronger is its acidic or bisic strength.

The equilibrium concept is not usually applied to stronger electrolytes. In case of strong electrolytes, the K approaches infinity, where amount of unionised matter is almost negligible.

In case of non-electrolytes, the K will approach zero, because amount of ionised substance approaches zero.

18. PRECIPITATION

If the main goal is quantitative precipitation, the whole process is known as gravimetry. In this procedure the measurement is weight or weight change. The following main chemical steps are involved in gravimetric analysis:

(a) The sample being analysed is weighed accurately.

(b) The weighed sample is dissolved in a suitable solvent.

(c) The substances which may interfere with actual measurement are generally removed by using suitable separation methods.

(d) The pH should be adjusted by using buffer solution.

(e) The experimental criterion is adjusted like change of oxidation state; concentration or diluting of the sample or adding of masking agents.

(f) A suitable inorganic precipitating reagent is added.

(g) Precipitation is carried out in hot dilute solution.

(h) The precipitate is then separated form the mother liquor by filtration.

(i) Precipitate is washed with sutiable solvent.

(j) The dried precipitate or some suitable precipitate product formed as a result of ignition is weighed finally.

Requirements for suitability of a reaction for use in chemical analysis

(a) The reaction should be stoichiometric.

(b) The chemical reaction should proceed fast, otherwise extra and excessive time would be required for the reaction to complete.

(c) The reaction should be quantitative and must be complete by 99.9%.

(d) After completing the reaction, it is necessary to know a convenient method to follow the progress of the reaction.

The reaction is assumed to be complete when one of the following takes place:

(i) Formation of undissociated molecules.

(ii) Formation of precipitate.

(iii) Formation of chelate.

(iv) Formation of gas.

ANALYTICAL BALANCE

Weighing is an integral part of almost any analysis, both for measuring the sample and for preparing standard solutions. In analytical chemistry the analyst deals with rather small weights, of the order of a few grams to a few milligrams or even less. Standard laboratory weighing is typically made to three or four significant figures, and so the weighing device must be both accurate and sensitive. There are various sophisticated ways of achieving this, but the most useful and versatile device used is analytical balance. Analytical balance gives the accurate measurement of mass. A chemical analyst really deals with mass rather than weight. Mass is the quantity of matter of which the object is composed and is invariant; on the other hand, the weight of an object is the force exerted on it by gravitational attraction. This force will differ at the different locations on the earth.

$$W = mg$$

When W is the weight of the object, m its mass and g is the acceleration due to gravity.

The weight of an object is variable whereas its mass is constant. It has become customery to employ the term 'weight' synonymously with mass, and it is in this sense that 'weight' is employed in quantitative analysis.

In recent years, analytical balance has undergone radical changes. The design of the balance has been altered, and the conventional free-swinging, equal-arm,two-pan chemical balance together with its box of weights is now an uncommon sight.

The following types of balance are described below:

(1) Electronic Balance

(2) Single-pan Balance

(3) Semimicro and Micro Balance

(1) Electronic Balance

Modern electronic balances offer convenience in weighing and are subject to fewer errors or mechanical failures than the mechanical balances. The operation of dialing weights, turning and reading micrometers, and beam and pan arrest of mechanical balances are eliminated, greatly speeding the measurement. A digital-display electronic balance is shown in Figure 6 and the operating principle of an electronic balance is illustrated in Figure 7. There

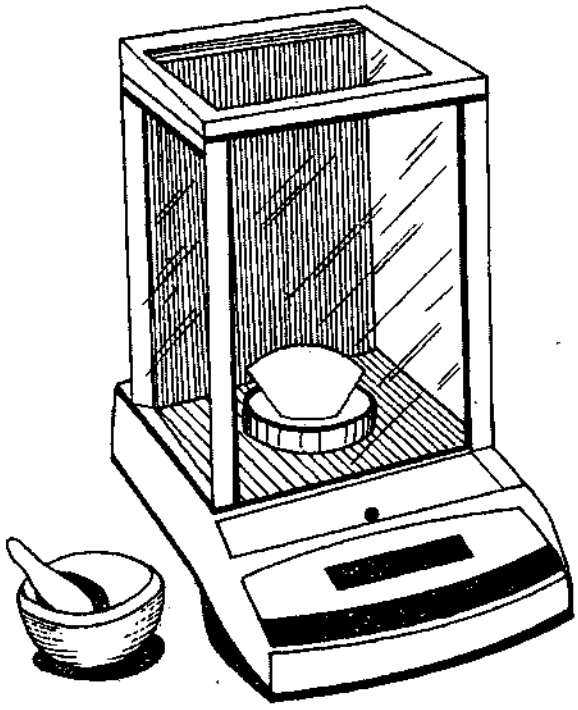


Fig. 6. Electronic analytical balance

are no weights or knife edges as in mechanical balances. The pan sits on the arm of a movable hanger (2), and this movable system is compensated by a constant electromagnetic force. The position of the hanger is monitored by an electrical position scanner (1), which brings the weighing system back to the zero position. The compensation current is proportional to the weight placed on the pan. This is sent in digital form to a microprocessor that converts it into the corresponding weight value, which appears as a digital display. The weight of the container can be automatically subtracted.

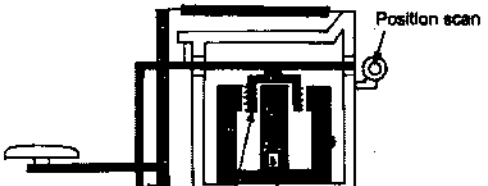


Fig. 7 Operating principle of electronic balance: 1. position scanner; 2. hanger; 3. coil; 4. temperature sensor

These balances use the principle of electromagnetic force compensation first described by Angstrom in 1895. But they still use the principle of comparing one weight with another. The balance is "zeroed", or calibrated, with a known weight. When the sample is placed on the pan, its weight is electronically compared with the known. This is a form of self-calibration.

Modern balances may have such features as compensating for wandering from true zero and averaging variations due to building vibrations.

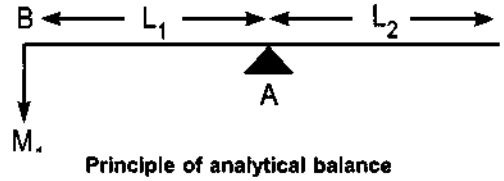
A single control bar is used to switch the balance on and off, to set the display to zero, and to trace a container automatically on the pan. Since results are available as an electrical signal, they can be readily processed by a personal computer and stored. Weighing statistics can be automatically calculated.

Electronic analytical balances can be purchased with different weighing ranges and readabilities. A macrobalance will have a range of the order of 160 g readable to 0.1 mg, and a semimicrobalance will have a range of about 30 g, readable to 0.01 mg. Microbalances weigh upto 1 μg, and ultramicrobalances are available that are sensitive to 0.1 μg or less.

Electrochemical quartz balances are available with 100-μg range that can detect 1 ng (10^{-9} g) changes. The balance utilizes a thin quartz crystal disk oscillating at, for example, 10 MHz. The frequency of oscillation changes with any change in mass, and the frequency change measured by the instrument is converted to mass units. A film of gold is evaporated on the quartz, and the gold substrate can be coated with the material of interest. Mass changes as small as a few percent of a monolayer coverage of atoms or molecules on the gold surface can be measured. Mass changes with time can be recorded.

(2) Single-pan Mechanical Balance

The mechanical analytical balance is a first-class lever that compares two masses. Figure 8 illustrates such a balance. The fulcrum A lies between the points of application of forces B and C. The term M_1 represents the unknown mass and M_2 represents a known mass. The principle of operation is based on the fact that at balance, $M_1L_1 = M_2L_2$. If L_1 and L_2 are made to be as nearly equal as possible, then, at balance, $M_1 = M_2$. A pointer is placed on the beam of the balance to indicate on a scale at the end of the pointer when a state of balance is achieved. The operator adjusts the value of M_2 until



Principle of analytical balance

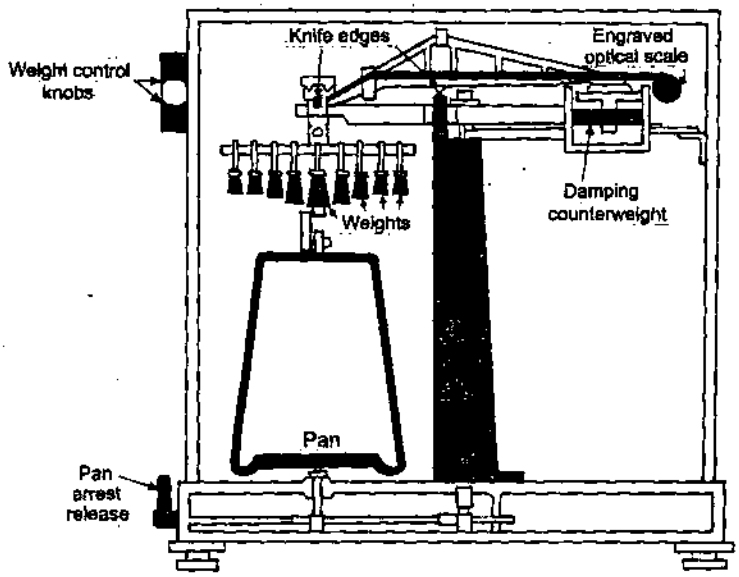


Fig. 8 Schematic diagram of a typical single-pan balance

the pointer returns to its original position on the scale when the balance is unloaded. Although mass is determined, the ratio of masses is the same as the ratio of weights when an equal-arm balance is used. It is customary, then, to use the term *weight* instead of mass and to speak of the operation as *weighing*. The known masses are called **standard weights.**

Most analytical weighings using mechanical balances are made on a singlepan balance. A schematic diagram of a typical mechanical single-pan balance is shown in Figure 8 (see below for a description of modern electronic balances).

A first-class (unsymmetrical) lever is pivoted on a knife edge, and a pan is kept at one end in which the object is placed. However, there is no pan at the other end for placing weights. When the balance is not in use, a series of weights totaling 160 to 200 g are on the pan end of the beam. These are counterbalanced by a single weight on the other end of the beam, which also acts as part of a damping piston. When an object is placed on the pan, individual weights are removed from this end of the beam to restore it to equilibrium. This is accomplished by means of knobs on the front of the balance that lift weights or combinations of weights from the beam. Thus, the weights are never handled. These weights will be equal to the weight of the object on the pan.

Actually, the beam is not brought completely to balance, but weights are removed only to the nearest whole gram or 0.1 g, depending on the balance. The imbalance of the beam is registered optically and automatically on an illuminated vernier scale by a light ray reflected from an engraved optical scale on the beam. The last digits (nearest 0.1 mg) are read from this scale. Alternatively, the imbalance may be read on a digital counter.

The original no-load reading or position is called the **zero point,** and the position under load is called the rest point. In operation, the **rest point** is made to coincide with the zero point. The zero point is generally adjusted to read zero by adjusting the vernier by means of a knob.

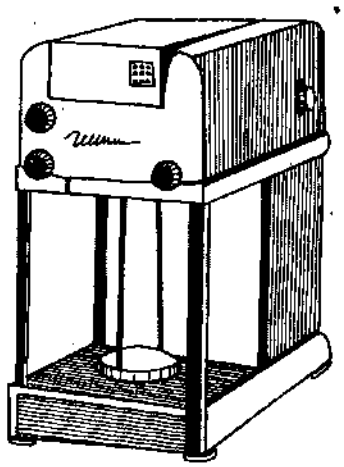


Fig. 9 Typical single-pan balance

Single-pan balances are under constant load of 160 or 200 g, a required feature since they are not brought back to a state of balance. The sensitivity of a balance varies with the load because it is governed by the center of gravity of the beam; the beam bends slightly under load, causing a change in the center of gravity and the sensitivity. Calibration of the vernier or digital readout of a single-pan balance to read the amount of imbalance is done at a given sensitivity, that is, at a given load. Therefore, the load must remain constant.

All weights of a single-pan balance are concealed and are removed by means of control knobs on the front of the balance: one for tens (*e.g.*, 10 to 90 g), one for units (1 to 9 g), and, if applicable, one for 0.1 units (0.1 to 0.9 g). The weights removed are registered on a counter on the front of the balance. The beam is brought to rest rapidly by means of an air piston damper.

Care must be taken not to damage the knife-edges while the balance is not in operation and while objects are being placed or removed from the pan. A

threeposition beam-arrest knob is used to protect the knife-edges and beam. The center position arrests the pan and beam; a second position partially releases the pan for use while finding the approximate weight of the object on the pan; and a third position completely releases the pan to allow the balance to come to rest.

A typical single-pan balance is shown in Figure. 9 Weighings can be made in less than a minute with these balances.

(3) Semimicro And Microbalances

Semimicro balance is sensitive to about 0.01 mg, and the microbalance is sensitive to about 0.001 mg (1 μg). The load limits of these balances are correspondingly less than conventional balance, and greater care must be taken in their use.

Care and use of analytical balances

It does not matter which type of analytical balance is employed, the following precautions should be taken in their use:

(1) Never exceed the stated maximum load of the balance.

(2) The balance must be kept clean. Remove dust from the pan and from the floor of the pan compartment with a camel-hair brush.

(3) Objects to be weighed should never be handled with fingers, always use tongs or a loop of clean paper.

(4) Objects to be weighed should be allowed to attain the temperature of the balance before weighing, and if the object has been heated, sufficient time must be allowed for cooling. The time required to attain the temperature of the balance varies with the size, etc. of the object, but as a rule 30-40 minutes time is sufficient.

(5) The chemical, which might injure the balance pan should never be placed directly on it. Substances must be weighed in suitable containers, such as, weighing bottles, crucibles, upon watch glasses or small beakers. Liquids and volatile or hygroscopic solids must be weighed in tightly closed vessels, such as stoppered weighing bottles. The addition of chemicals to the receptacle must be done outside the balance case.

(6) When weighing has been completed, nothing must be left on the pan.

(7) The exposure of the balance to corrosive atmosphere must be avoided.

(8) Keep the object on the pan, when balance is at rest.

(9) When the balance is linked to a printer then confirm that the printed result agrees with the digital display.

(10) When all weighings have ben completed, remove the object which has been weighed, clean the pan and close the pan compartment.

ERRORS IN WEIGHING

There may be errors in weighing, even when done very carefully, due to the following reasons :

(1) Changes in the conditions of the containing vessels of the substances between successive weighings.

(2) Errors in recording the weights.

(3) Effect of the buoyancy of air upon the object and the weights.

(1) Changes in the conditions of the containing vessels : The first source of error is occasioned by change in weight of the containing vessel: (a) by absorption or loss of moisture

(b) by electrification of the surface caused by rubbing

(c) by its temperature being different from balance case

These errors may be eliminated by wiping the vessel gently with a linen cloth and allowing it to stand at least for 30 minutes in proximity to the balance before weighing.

The electrification may be removed by subjecting the vessel to the discharge from an antistatic gun.

Hygroscopic, efflorescent, and volatile substances must be weighed in completely closed vessels.

Substances which have been heated in an air oven or ignited in a crucible should generally be allowed to cool in a desiccator containing a suitable drying agent.

(2) Errors in recording the weights: The correct reading and recording of weights is best achieved by checking weights as they are added to the balance and as they are removed from the balance. In the case of electronic balances any digital display should be read at least twice.

(3) Effect of buoyancy of the air: When a substance is immersed in a fluid, its true weight is diminished by the weight of the fluid which it displaces. If the object and the weights have the same density, and the same volume, then no error will be introduced on this account. If the density of the object is different from that of the weights, the volume of air displaced by each will be different. If the substance has a lower density than the weights, as is usual in analysis, the former will displace a greater volume of air than the latter, and it will, therefore, weigh less in air than in vacuum. Conversely, if a denser material (*e.g.*, one of the precious metals) is weighed, the weight in vacuum will be less than the apparent weight in air.

CLEANING OF GLASS WARES

Glass ware must be perfectly clean and free from grease, otherwise the result will be unreliable. One test for cleanliness of glassware is that upon being filled with distilled water and the water withdrawn, only an unbroken film of water remains. If the water collects in drops, the vessel is dirty and must be cleaned.

Various methods are available for cleaning of glassware:

(1) Decon 90: Many commercial detergents are available which are suitable for this purpose. Some manufacturers market special formulations for cleaning laboratory glassware. Some of these *e.g.*, Decon '90' made by Decon Laboratories of Portslade is found to be specially effective in removing contamination due to radioactive materials.

(2) Teepol: Teepol is relatively mild and inexpensive detergent which may be used for cleaning glassware. The laboratory stock solution may consist of a 10 percent solution in distilled water. For cleaning a burette, 2 mL of the stock solution diluted with 50 mL of distilled water is poured into the burette, it is allowed to stand for 1/2 minute to 1 minute, the detergent is run off, the burette is rinsed three times with tap water, and then several times with distilled water. A 25 mL pipette may be similarly cleaned using 1 mL of the stock solution diluted with 25-30 mL of distilled water.

(3) CARE: A method which is frequently used, consists of filling up the apparatus with 'Chromic acid cleaning mixture' (CARE), a nearly saturated solution of powdered sodium dichromate or potassium dichromate in

concentrated sulphuric acid, and allowed to stand for several hours, preferably overnight, The acid is then poured off, the apparatus thoroughly rinsed with distilled water and allowed to drain until dry. [Potassium dichromate is not very soluble in concentrated sulphuric acid (about 5g per litre), whereas sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) is much more soluble (about 70g per litre); for this reason, as well as the fact that it is much cheaper, the latter is usually preferred for the preparation of 'Cleaning mixture'.] From time to time it is advisable to filter sodium dichromate-sulphuric acid mixture through a little glass wool placed in the apex of glass funnel. Small particles of sludge, which are often present and block the tip of burettes, are thus removed.

(4) Mixture of Sulphuric Acid-Nitric Acid: A more efficient cleaning liquid is a mixture of concentrated sulphuric acid and fuming nitric acid. This may be used when the vessel is very greasy and dirty, but it must be handled with extreme caution.

(5) Mixture of KOH-Spirit: A very effective and degreasing agent, which is claimed to be much quicker-acting than 'cleaning mixture' is obtained by dissolving 100 g of potassium hydroxide in 50 mL of water and after cooling making up to 1 litre with industrial methylated spirit.

CALIBRATION OF GLASSWARE

For most of the analytical purposes, volumetric apparatus manufactured to class A standard will prove satisfactory, but for work of the highest accuracy it is advisable to calibrate all apparatus for which a recent test certificate is unavailable. The calibration procedure involves determination of the weight of water contained or delivered by the particular piece of apparatus. The temperature of watet is observed, and from the known density of water at that temperature, the volume of water can be calculated.

The volume of 1g of water at various temperatures is given below, which can be used to calculate the relevant volume directly from the observed weight of water.

Temperature (°C)	Volume (mL)	Temperature (°C)	Volume (mL)
10.00	1.0013		
12.00	1.0015	22.0	1.0033
14.00	1.0017	24.0	1.0037
16.00	1.0021	26.0	1.0044
18.00	1.0023	28.0	1.0047
20.00	1.0027	30.0	1.0053

PREPARATIONS BEFORE CALIBRATION OPERATION

In all calibration operations, apparatus to be calibrated must be carefully cleaned and allowed to stand adjacent to the balance which is to be used, together with the supply of distilled or de-ionised water, so that they acquire the temperature of the room. Flasks will also need to be dried, and this can be accomplished by rinsing twice with a little acetone and then blowing a current of air through the flask to remove the acetone.

Graduated Flask: After allowing the clean dry flask to stand in the balance room for an hour it is stoppered and weighed. A small filter funnel, the stem of which has been drawn out so that it reaches below the graduation mark of the flask, is then inserted into the neck and distilled water which has also been standing in the balance room for an hour, is added slowly until the mark is reached. The funnel is then carefully removed, taking care not to wet the neck of the flask above the mark, and then, using a dropping tube, water is

added dropwise until the meniscus stands on the graduation mark. The stopper is replaced, the flask is reweighed, and the temperature of water noted. The true volume of the water filling the flask to the graduation mark can be calculated with the help of data given above.

Pipette: The pipette is filled with distilled water (which has been standing in the balance room for at least an hour) above the mark. Now remove the excess of water and bring the meniscus at the mark. There should be no droplets on the surface, if there are, blot them with tissue paper. The pipette is then allowed to discharge into a clean weighed stoppered flask and held so that the tip of the pipette is in contact with the side of the flask. The pipette is allowed to drain for 15 seconds after the out-flow has ceased. The receiving flask is removed from contact with the tip of the pipette. The receiving flask is weighed and the temperature of the water noted. The capacity of the pipette is then calculated with the help of the data given above. At least two determinations of the whole process should be made.

Burette: The calibration of a burette is similar to the procedure for a pipette, except that several volumes will be delivered.

To calibrate a burette, first make satisfaction about its (a) leakage and (b) delivery time

(a) Leakage: To test the leakage, the plug is removed from the barrel of the stop cock and both parts are carefully cleaned of all grease, after wetting well with distilled water the stop cock is reassembled. The burette is placed in the holder, filled with distilled water, adjusted to the zero mark, and any drop of water adhering to tip is removed with the tissue paper or filter paper. The burette is then allowed to stand for 20 minutes, and if the meniscus has not fallen by more than one scale division, the burette may be regarded as satisfactory as far as leakage is concerned.

(b) Delivery time: To test the delivery time, again separate the components of the stopcock, dry, grease and reassemble, then fill the burette upto zeromark with distilled water, and place in the holder. Open the stopcock fully and note the time taken by the meniscus to reach the lowest graduation mark of the burette, this should agree with the time marked on the burette.

After passing above two tests, the burette should be ready for calibration operation. First fill the burette with distilled water (which has been kept in the balance room to acquire room temperature). Weigh a clean dry stoppered flask of 100 mL capacity. Adjust the burette to the zero mark, blot any water droplet adhering to the tip of burette, place the flask in position under the tip of the burette, open the stopcock fully, and allow the water to flow into the flask. As the meniscus approaches the desired calibration point on the burette, reduce the rate of flow until eventually it is discharging dropwise, and adjust the meniscus exactly to the required mark. Repeat this procedure for each graduation to be tested; For a 50 mL burette, this will usually be every 5 mL. Note the temperature of the water and then using above data, the volume delivered at each point is calculated from the weight of water collected. The calibration results will be more conveniently used by plotting a curve for the burette.

SAMPLE PREPARATION

WEIGHING THE SAMPLE

The material is usually dried at 105 ⊃ C - 110 ⊃ C before analysis. After

cooling in a desiccator, the material is transferred to a weighing bottle which is stoppered and stored in a desiccator. Samples of appropriate size are withdrawn from the weighing bottle as required, the bottle being weighed before and after the withdrawal, so that the weight of substance is obtained by difference.

SOLUTION OF THE SAMPLE

Most organic substances can be dissolved readily in a suitable organic solvent and some are directly soluble in water or can be dissolved is aqueous solutions of acids (basic materials) or of alkalis (acidic materials). Many inorganic substances can be dissolved directly in water or in dilute acids, but materials such as minerals refractories, alloys must usually be treated with a variety of reagents in order to discover a suitable solvent.

For a substance which dissolves readily, the sample is weighed out into a beaker, and the beaker is immediately covered with a clockglass of suitable size (its diameter should not be more than about 1 cm larger than that of the beaker) with its convex side facing downwards. The beaker should have a spout in order to provide an outlet for the escape of steam or gas. The solvent is then added by pouring it carefully down a glass rod, the lower end of which rests against the wall of the beaker, the clockglass is displaced somewhat during this process. If a gas is evolved during the addition of the solvent (*e.g.*, acids with carbonates, metals, alloys etc.) the beaker must be kept covered as far as possible during the addition. The reagent is then best added by means of a pipette or by means of a funnel with a bend stem inserted beneath the clockglass at the spout of the beaker; loss by spirting or as spray is thus prevented. When the evolution of gas has ceased and the substance is completely dissolved, the under side of the clockglass is well rinsed with a stream of water from a wash bottle, care being taken that the washings fall on the side of the beaker and not directly in the solution. If warming is necessary, it is usually best to carry out the dissolution in a conical flask with a small funnel in the mouth, loss of liquid by spirting is thus prevented and the escape of gas is not hindered. When using volatile solvents, the flask should be fitted with reflux condenser.

To reduce the volume of the solution or sometimes to evaporate completely to dryness, wide and shallow vessels are most suitable, because a large surface is thus exposed and evaporation is thereby accelerated. Evaporation should be carried out on the steam bath or upon a low temperature hot plate. During evaporation, the vessel must be covered by a pyrex clock glass, slightly larger in diameter than the vessel. At the end of the evaporation the sides of the vessel, and the lower side of the clockglass should be rinsed with distilled water into the vessel.

DISSOLVING THE INORGANIC SOLIDS

Strong mineral acids are good solvents for many inorganics. Hydrochloric acid is a good general solvent for dissolving metals, which are above hydrogen in the electromotive series. Nitric acid is a strong oxidizing agent and dissolves most of the common metals, nonferrous alloys, and the "acid insoluble" sulphides.

Perchloric acid, when heated to drive off water, becomes a very strong and efficient oxidizing acid in the dehydrated state. it dissolves most of the common metals and destroys organic matter. It must be used with extreme

caution because it will react explosively with many easily oxidizable substances specially organic matter. Some inorganic materials will not dissolve in acids, and fusion with an acidic or basic flux in the molten state must be employed to render them soluble.

SAMPLE DECOMPOSITION AND DISSOLUTION

Most of the analytical determinations are performed in aqueous solutions of the analyte. While some samples dissolve rapidly in water or aqueous solutions of the common acids or bases, other samples require strong reagents and treatment to dissolve them. For example:

(a) To destroy the silicate structure of a siliceous mineral and to free the ions for anlysis, the sample requires high temperature and pressure, and many other drastic conditions.

(b) The compounds of halogen and sulphur are treated at high temperatures and with powerful reagents to rupture the strong bonds between elements and carbon atom. In this way it becomes possible to determine the sulphur and halogen elements in that organic compound.

It is very difficult to select a proper reagent and technique for decomposing and dissolving an analytical sample particularly when refractory substance is involved or the sample is present in trace quantity.

There are four types of common methods of decomposing and dissolving an analytical sample.

(1) Heating with aqueous strong acids or bases in open vassel.

(2) Microwave heating with acids.

(3) High temperature ignition in air or oxygen.

(4) Fusion in molten salt media.

These methods differ in the temperature at which they are carried out and the strength of the reagents used.

(1) DECOMPOSING SAMPLES WITH INORGANIC ACIDS IN OPEN VESSELS

The mineral acids are generally used to decompose the inorganic samples in open-vessel. Sometimes ammonia and aqueous solutions of the alkali metal hydroxides are used for this purpose. Usually, a suspension of the inorganic sample is prepared in the mineral acid and then it is heated by flame or a hot plate until the dissolution is conformed to be complete by the total disappearance of a solid phase. In this process the temperature of decomposition is adjusted at the boiling point of the used acid reagent.

The following acids can be used for the purpose, :

(a) Hydrochloric Acid

(b) Nitric Acid

(c) Sulphuric Acid

(d) Perchloric Acid

(e) Oxidising Mixture of acids

(f) Hydrofluoric acid.

(a) Hydrochloric Acid : Concentrated HCl is an excellent solvent for inorganic samples. It is widely used to dissolve the metals which are more easily oxidized than hydrogen as well as many metal oxides. Hydrochloric acid has limited applications in decomposition of organic substances. The strength of concentrated HCl is about 12 M.

(b) Nitric Acid : To dissolve all common metals except aluminium and chromium (owing to sufrace oxide formation), hot concentrated nitric acid is a potent oxidant. For the determination of the trace metal contents in samples, hot nitric acid is used alone or in combination with other acids and oxidizing agents. For decomposition of organic samples, hot nitric acid is used with hydrogen peroxide and bromine. In this process organic sample converts into CO_2 and water. This decomposition process is known as wet ashing process.

(c) Sulphuric Acid : Many materials are decomposed and dissolved by hot concentrated H_2SO_4. As the boiling point of sulphuric acid is very high (about 340°C), most of the organic compounds are dehydrated and oxidized at this temperature and thus eliminated from the sample as CO_2 and H_2O by this wet ashing process.

(d) Perchloric Acid : Perchloric acid is a very strong acid which can attack even on iron alloys and stainless steel (which are not affected by other mineral acids). Due to its high explosive nature, perchloric acid is used with great care. Although the dilute heated acid and cold concentrated acid is not explosive in nature, violent explosion occurs when hot concentrated perchloric acid comes in contact with organic materials or easily oxidised inorganic substances. Because of this property, this acid is heated in a special hood, lined with glass or stainless steel and its own fan system. The hood for perchloric acid should be independent of all other systems. Commercially perchloric acid is marketed as 60% to 72% acid.

(e) Oxidising Mixture of acids : To decompose the samples, some oxidising agents are used as the mixture of acids, such as:

(i) Aquaregia, a mixture containing 3 volumes of concentrated HCl and 1 volume of nitric acid.

(ii) The addition of bromine or hydrogen peroxide to mineral acids generally increases their solvent action.

(iii) Mixtures of nitric acid and perchloric acid are also useful for this purpose and are less dangerous than perchloric acid alone.

(f) Hydrofluoric Acid : The main use of hydrofluoric acid is for the decomposition of silicate rocks and minerals in the analysis of elements other than silica. Hydrofluoric acid finds occasional use with other acids in dissolving steel which is difficult to dissolve in other solvents. As hydrofluoric acid is extremely toxic, therefore dissolution of samples and evaporation to remove excess reagent should always be carried out in a well-ventilated hood. Hydrofluoric acid causes serious damage and painful injury when brought in contact with skin. If the acid comes in contact with skin, the affected area should be immediately washed with large quantity of water.

(2) MICROWAVE DECOMPOSITION

The use of microwave ovens for the decomposition of organic and inorganic compounds was first proposed in 1970. Microwave digestions can be carried out in both closed as well as open vessels, but closed vessels are often used because higher pressure and temperature can be achieved in them.

The decomposition of compounds in microwave oven is very fast and speedy, even in case of typical and difficult samples it takes only 5 to 10 minutes. In contrast, the same results require several hours when sample is heated over a flame or a hot plate.

In Conventional Method: The heat transfer is by conduction, because the vessels used in conductive heating are usually poor conductors, time is required to heat the vessel and then transfer the heat to the solution by conduction.

In Microwave Oven: The energy is transferred directly to all the molecules of the solution almost simultaneously without heating up the vessel. Thus, boiling temperatures are reached throughout the entire solution very quickly.

An advantage of using closed vessels for microwave decomposition is the higher temperature that develops as a consequence of increased pressure. A further advantage of micro-wave decomposition is that loss of volatile components of samples is virtually eliminated. Closed vessel microwave decomposition is often easy to automate, thus reducing operation time required to prepare samples for analysis.

Moderate Pressure Vessels : Microwave digestion vessels are constructed from low loss materials. These vessels are transparent, thermally stable, resistant to chemical attack by various acids used for decomposition. The best material for microwaves is teflon. It is transparent, inert against sulphuric acid and phosphoric acid, having a melting point about 300°C. Quartz or borosilicate glass vessels are sometimes used in place of teflon containers. A closed digestion vessel of teflon body consists of a cap and a safety relief valve to operate at 120 + 10 Psi pressure is used commercially in a microwave oven. At this pressure, the safety valve can open and then resealed (Fig. 10).

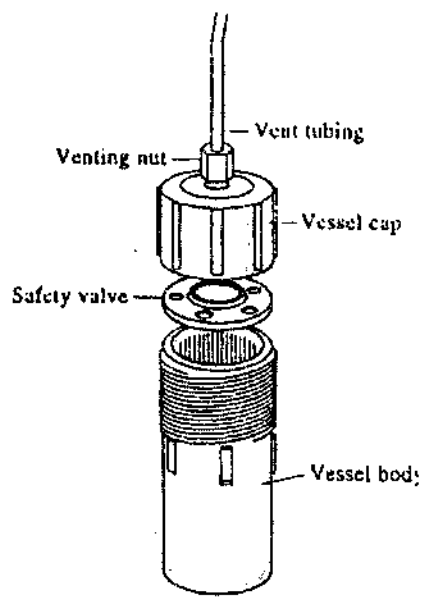


Fig. 10 A moderate-pressure vessel for microwave decomposition

High Pressure Microwave vessels: For dissolving highly refractory materials which are incompletely decomposed in the moderate pressure vessels, the microwave bomb is particularly useful. In this bomb, the heavy-wall body is composed of a polymeric material which is transparent to microwaves. The decomposition of the material is carried out in a teflon cup supported in the bomb body. It has a teflon O–ring in the liner cap that seats against a narrow rim on the exterior of the liner. The O–ring distorts, and the excess pressure then compresses the sealer disk, which allows the gases to escape into the surroundings. The internal pressure in the bomb can be judged by the distance to which pressure screw protrudes from the cap. A commercial Microwave bomb is designed to operate at 80 atm. or about 10 times the pressure that can be tolerated by the moderate pressure vessels as described above (Fig. 11).

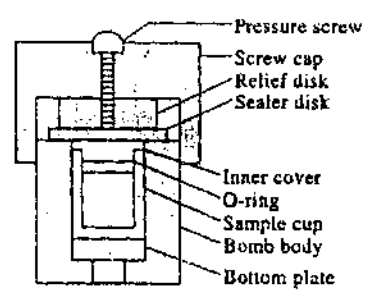


Fig. 11 A bomb for highpressure microwave decomposition

Atmospheric Pressure Vessels : Atmospheric pressure Vessel are open-vessel systems. These systems do not have an oven but use a focussed micro-wave cavity. They have tubing for the insertion and removal of reagents. There is no safety because of formation of gas during the reaction of digestion process since the systems operate at atmospheric pressure.

Microwave Ovens : In a microwave oven, 12 moderate pressure vessels can be heated at a time. It is designed as given in Fig. 12. The vessels can rotate continuously through 360 degrees so that the average energy received by each vessel is approximately the same.

Microwave Furnaces : In microwave furnace an organic material can be fused before acid dissolution. These furnaces consist of a small volume chamber which accomodates only an ordinary-sized crucible. This small cavity-like chamber is made up of silicon carbide which is surrounded by quartz insulation. The advantage of this type of furnace over a conventional muffle furnace is the speed, at which the high temperature (1000°C) is reached in 2 minutes. In contrast, the muffle furnaces are generally operated continuously because of the time required to get them upto required temperature. In a microwave furnace there is no burnt out heating coils as frequently encountered in the conventional furnaces.

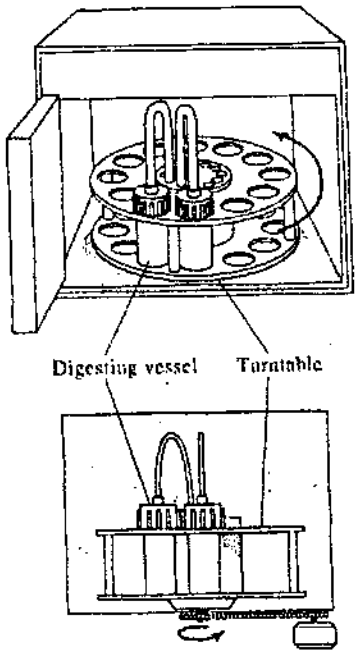


Fig. 12 A microwave oven designed for use with 12 vessels of the type

Applications of Microwave Decomposition

The applications of closed vessel decomposition in microwave oven fall into two categories:

(i) Oxidative decomposition of organic and biological samples.

(ii) Decomposition of refractory inorganic materials in industry.

In both the above cases, the new microwave technique is replacing older conventional methods because of the large economic gains that result from significant saving in time.

(3) COMBUSTION METHODS FOR DECOMPOSING ORGANIC SAMPLES

(a) Combustion over an open Flame (Dry Ashing): To determine the cations of an organic sample, it has to be heated till red hot in an open dish or crucible over a flame so that all the carbonaceous matter gets oxidized and converted into carbondioxide. Analysis of the non-volatile components follows dissolution of the residual solid. In addition, volatile metallic compounds may be lost during the ignition process. Although dry ashing is the simplest method of decomposing organic compounds, it is often the least reliable.

(b) Combustion-Tube method: Several common and important elemental components of organic compounds are converted into gaseous products as a sample is pyrolyzed in the presence of oxygen. The heating is commonly performed in a glass or quartz combustion tube through which a stream of carrier gas is passed. The stream transports the volatile products to the parts of the apparatus where they are separated and retained for

measurement. Elements susceptible to this type of treatment are carbon, hydrogen, nitrogen, sulphur, halogens and oxygen.

Automated combustion-tube analyzers are now available for the determination of either carbon, hydrogen, and nitrogen or carbon, hydrogen and oxygen in a sample. This analysis is completed in less than 15 minutes. In this analyzer, the sample is ignited in a stream of helium and oxygen and passed over an oxidation catalyst consisting of a mixture of silver vandate and silver tungstate. In this process the halogens and sulphur are removed with a packing of silver salts. A packing of hot copper is located at the end of combustion train to remove oxygen and convert nitrogen oxides to nitrogen. The exit gas, consisting of a mixture of H_2O, CO_2, nitrogen and helium is collected in a glass bulb.

(c) Combustion with oxygen in a sealed container : The decomposition of many organic materials involves sealed container. Before the reaction vessesl is opened, the reaction products are absorbed in a proper solvent. Schoniger suggested an apparatus (Fig. 13). It contains a heavy-walled flask of 300-1000 mL capacity fitted with a ground glass stopper. Attached to the stopper is a platinum gauze basket which holds 2 to 200 mg of sample. If the substance is a solid, it is wrapped in a piece of low-ash filter paper. The liquid samples are weighed in a gelatin capsule which is then wrapped in a similar manner. The paper tail helps as the ignition point. A small volume of an absorbing solution is taken in the flask. The tail of the paper is ignited, the stopper is fitted rapidly into the flask, and the flask is inverted to prevent the escape of the volatile oxidation products. The entire reaction is calalyzed by the platinum gauge surrounding the sample. This procedure is helpful to determine the halogens, sulphur, phosphorus, fluorine, boron, carbon, arsenic, and various other metals present in organic compouds.

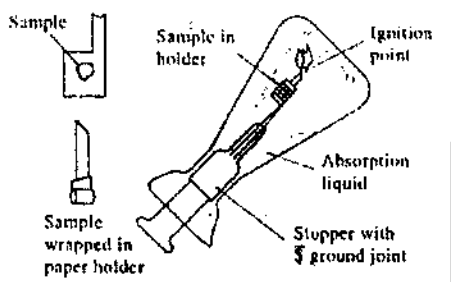


Fig. 13 Schöniger combustion apparatus

(4) DECOMPOSITION OF INORGANIC MATERIAL BY FLUXES

Some substances can not decompose easily such as some mineral oxides, silicates and some iron alloys. In such cases fused-salt medium is mixed with the sample and then this combination forms a water–soluble product called melt.

Flux is an alkali-metal salt, used to decompose the substances which are attacked by the reagent very slowly.

For a sample containing a small fraction of substance which dissolves with difficulty, is mixed with a liquid reagent, the undecomposed residue is then separated by filtration and is fused with a small quantity of proper flux. After cooling the above combination, the melt is dissolved and combined with the major fraction of the sample.

Procedure of Fusion: Firstly, the sample is prepared by grinding and fine powder is obtained. It is mixed with a suitable flux. The quantity of flux should be 10 times of the sample. Mixing of flux and sample is carried out in a crucible. The fusion is done in the crucible for a few minutes to a few hours. The formation of a clear melt indicates the completion of decomposition. After completing the fusion, the mass is allowed to cool, before solidification.

Types of Fluxes: The common fluxes used in analysis are compounds of the alkali metals.

The fluxes are of following types:

(a) Basic Fluxes: These are alkali metal carbonates, hydroxides, peroxides, and borates. The basic fluxes are used to attack acidic materials.

(b) Acidic Fluxes: These are pyrosulphates, acid fluorides, and boric oxide.

(c) Oxidizing Fluxes: *e.g.*, sodium peroxide.

The properties of some common fluxes are given below:

(i) Sodium Carbonate: With sodium carbonate the sample containing silicates and some other refractory materials can be decomposed by heating at 1000°C to 1200°C. By this fusion, the cationic constituents of the substance (sample) generally convert into acid-soluble carbonates or oxides and non-metallic constituents are converted into soluble sodium salts. Carbonate fusion is usually performed in platinum crucible.

(ii) Potassium Pyrosulphate: Potassium pyrosulphate is a strong acidic flux which is used for attacking the more intractable metal oxides.

With it flux fusion is carried out at 400°C.

Potassium pyrosulphate can be prepared by heating potassium hydrogen sulphate, in this way:

$$2KHSO_4 \longrightarrow K_2S_2O_7 + H_2O$$

(iii) Lithium Metaborate: $LiBO_2$ [lithium metaborate], is mixed with lithium tetraborate, and fused with refractory silicate and alumina minerals. This fusion is carried out in platinum or graphite crucible at 900°C.

Common Fluxes

Flux	Melting Point, °C	Type of Crucible for Fusion	Type of substance Decomposed
Na_2CO_3	851	Platinum	Silicates and silica containing samples, alumina containing samples, sparingly soluble phosphates and sulfates
Na_2CO_3 + anoxidizing agent e.g., KNO_3 or $KClO_3$ or Na_2O_2	–	Platinum or Nickel	Samples containing S, Sb, As, Cr, etc.
$LiBO_2$ (Basic Flux)	849	Platinum, gold, glassy carbon	For silicates, slags, ceramics,
NaOH or KOH (Basic Flux)	318 380	Gold, Nickel, Silver	For silicates, silicon carbide, certain minerals.
Na_2O_2 (Basic oxidizing Flux)	decomposes	Iron, Nickel	For sulfides, acid insoluble alloys of Fe, Ni, Cr, Mo, W, Li platinum alloys, Cr, Sn, Zr minerals
$K_2S_2O_7$ (Acidic Flux)	300	Platinum porcelain	For slightly soluble oxides and oxide containing samples

B_2O_3 (Acidic Flux)	577	Platinum	For silicates, oxides where alkali metals are to be determined
$CaCO_3 + NH_4Cl$	–	Nickel	For silicates to determine alkali metals

GRAVIMETRIC TECHNQUES

Gravimetric Analysis :

Gravimetric analysis or quantiative analysis is the process of isolating and weighing an element or a definite compound of the element in as pure a form as possible. The element or compound is separated from the weight portion of the substance which has to be examined. A large proportion of the determinations in gravimetric analysis is concerned with the transforamtion of the element or radical to be determined into a pure stable compound which can be readily converted into a form suitable for weighing. The weight of the element of redical may be readily calculated from formula of the compound and the relative atomic masses of the constitutent elements.

The separation of the element or compound by be effected in a number of ways, the moist important of which are : (a) precipiation method; (b) volatilsation or evolution method; (c) electronalytical method; and (d) extraction and chromatographic method.

The gravimetric anaolysis is generally somewhat time-consuming. The advantages of gravimetric analysis are :

(a) it is accurate and precise when using modern analytical balances;

(b) possible sources of error are readily checked and filtrates can be tested for completeness of precipitation and precipitates may be examined for the presence of impurities;

(c) it has the important advantage of being an absolute method;

(d) determinations can be carried out with relatively inexpensive apparatus, the most expensive requirements being a muffle furnace and, in some case platinum crucibles.

Two general applications of gravimetric analysis are :

(a) the analysis of standards which are to be used for the testing and calibration, of instrumental techniques;

(b) analysis requiring high accuracy, although the time-consuming nature of gravimetry limits this application to small number of determinations.

Precipitation Method :

This is the most important method in gravimetric analysis. The constituent being determined is precipitated from solution in a form which is slightly soluble, and the precipitate is separated by filtration and weighed. Thus, in the determination of silver, a solution of the substance is treated with an excess of sodium chloride or potassium chloride solution, the precipitate is filtered off, well washed to remove soluble salts dried at 130-150°C, and weighed as silver chloride. Frequently, the constituent being determined is weighed in a form other than that in which it was precipitated. Thus

magnesium is precipitated, as ammonium magnesium phosphate $Mg(NH_4)PO_4, 6H_2O$, but is weighed, after ignition, as pyrophosphate $Mg_2P_2O_7$. The following factors determine a successful analysis by precipitation method :

1. The precipitate must be so insoluble that no appreciable loss occurs when it is collected by filtration.

2. The physical nature of the precipitate must be such that it can be readily separated from the solution by filtration, and can be washed free of soluble impurities. These conditions require that the particles are of such size thi they do not pass through the filtering medium, and the particle size 8 unaffected by the washing process.

3. The precipitate must be convertible into a pure substance of defini chemical composition; this may be effected either by ignition or by a simp chemical operation, such as evaporation, with a suitable liquid.

It is assumed that the compound which separated out from the solution is chemically pure, but this is not always the case. The purity of the precipitate depends upon the substances present in solution before and after the addition of the reagenl and also upon the exact experiemntal conditions of precipitation.

Problems which arise with certain precipitates include the coagulation or flocculation of a colloidal dispersion of a finely divided solid to permit its filtration and to prevent its re-peptisation upon washing the precipitate. It is, therefore, desirable to understand the basic principles of the colloidal state relevant to quantative analysis as given below :

The Colloidal State

The colloidal state of matter is known by a certain range of particle size. Before discussing these, the various units which are employed in expressing small dimensions are given below :

$$1 \text{ pm} = 10^{-3} \text{ mm}; \; 1 \text{ nm} = 10^{-6} \text{ mm};$$

$$1 \text{ Angstrom unit} = \text{Å} = 10^{-10} \text{ meter} = 10^{-7} \text{ mm} = 0.1 \text{ nm}.$$

Colloidal properties are exhibited by substances of particle size ranging between 0.1 μm and | nm. Ordinary quantitative filter paper will retain particles down to a diameter of about 10^{-2} mm or 10 pm, so that colloidal solutions behave like true solutions and are not filterable (size of molecules is of the order of 0.1 nm or 10^{-8} cm). The limit of vision under the microscope is about 0.2 μm. If a powerful beam of light is passed through a colloidal solution and the solution is viewed at right angles to the incident light, a scattering of light is observed. This is the so-called Tyndall effect, which is not exhibited by true solutions.

An important consequence of the smallness of the size of colloidal particles is that the ratio of surface area to weight is extremely large. Phenomenon, such as adsorption, which depends upon the size of the surface, therefore, plays an important part with substances in the colloidal state.

Colloids are divided into two main groups, designated as lyophobic and lyophilic colloids. The properties of each group are described below :

	Lyophobic colloids		Lyophilic colloids
1.	The dispersion (or sols) are only slightly	1.	The dispersions are very viscous; they set viscous. Examples : sols of metals, silver to . jelly-like masses known as_ gels. halides, metallic sulphides, etc. Examples : sols. of silicic acid, tin (IV) oxide, -elatin.
2.	A comparatively minute concentration of an electrolyte results in flocculation. The change is, irreversible; water has no effect upon the flocculated solid.	2.	Comparatively large concentrations of electiolytes are required to cause precipitation ('salting out'). the change is reversible, and reversal is effected by the addition of a solvent (water).
3.	Lyophobic colloids, have an electric charge of definite sign, which can be changed only by special methods.	3.	Most lyophilic colloids change their charge readily, e.g, they are positively charged in an acid medium and negatively char'ed in an alkaline medium
4.	The ulua-microscope reveals bright particles in vigorous motion (Brownian movement),	4.	Under a diffuse light cone is exhibited under the ultra microscope.

The process of dispersing a gel or a flocculated solid to form a sol is **called Peptisation.**

The stability of lyophobic colloids is intimately associated with the electrics charge on the particles. Thus in the formation of an arsenic (III) sulphide sol by precipitation with hydrogen sulphide in WH acid solution, sulphide ions are primarily adsorbed (since every precipitate has a tendency to adsorb its own ions), and some hydrogen ions are secondarily adsorbed. The hydrogen particle ions or other ions which are secondarily adsorbed have been termed 'counter-ions'. Thus the so-called electrical double layer is set up between the particles and the solution. The colloidal particle of arsenic (III) sulphide has a negatively charged surface, with positively charged counter-ions which impart a positive charge to the liquid immediately surrounding it. If an electric current is passed through the solution, the negative particles move towards the anode; the speed is comparable with that of electrolytic ions. The electrical conductivity of a sol is quite low because the number of current-carrying particles is small compared with that in a solution of an electrolyte at an appreciable concentration; the large charge carried by the colloidal particles is not sufficient to compensate for their smaller number.

If the electrical double layer is destroyed, the sol is no longer stable, and the particles will flocculate, thereby reducing the large surface area. Thus, if barium chloride solution is added, barium ions are adsorbed by the particles; the charge distribution on the surface is disturbed and the particles flocculate. After flocculation,) it is found that the dispersion medium is acid owing to the liberation of the hydrogen counter-ions. It is supposed that ions of opposite

charge which adsorbed on the surface are necessary for coagulation. The minimum amount of electrolyte necessary to cause flocculation of the colloid is called the **flocculation** or **coagulation value**.

If two sols of opposite sign are mixed, mutual coagulation occurs causing the neutralisation of charges in lyophobic colloids. Lyophilic colloids are generally more difficult to coagulate than lyophobic colloids. If a lyophilic colloid, e.g. of gelatin, is added to a lyophobic colloid, e.g. of gold, then the lyophobic colloid appears to strongly protected against the flocculating action of electrolytes. It is probable that the particles of the lyophilic colloid are adsorbed by the lyophobic colloid and impact their own properties to the latter. The lyophilic colloid is known as a protective colloid. This explains the relative stability caused by the addition of a gelatin to th unstable gold sols. For this reason organic matter, which might form a protective colloid, is generally destroyed before proceeding with an inorganic analysis.

During the flocculation of a colloid by an electrolyte, the ions of opposite sig" of the colloid are adsorbed to a varying degree on the surface; the higher the charge of the ion, the more strongly it is adsorbed. In all cases, the precipitate will contaminated, by surface adsorption. On washing the precipitate with water, small part of adsorbed electrolyte is removed therefore precipitates are always washed with a suitable solution of an electrolyte which does not interfere with the subsequent steps in the determination.

Supersaturation and Precipitate Formation

The solubility of a substance at any given temperature in a given solvent is the amount of the substance dissolved by a known weight of that solvent when the substance is in equilibrium with the solvent. The solubility depends upon the particle size. A supersaturated solution is one that contains a greater concentration of solute than that which corresponds to the equilibrium solubility at the temperature under consideration. Supersaturation is, therefore, an unstable state which may be brought to a state of stable equilibrium by the addition of a crystal of the solute ('seeding' the solution) or of some other substance, or by mechanical means such as shaking or stirring.

Supersaturation plays an important part in determining the particle size of a precipitate. The initial velocity of precipitation is proportional to $(Q - S)/S$, where Q is the total concentration of the substance that is to precipitate, and S is the equilibrium solubility; $(Q - S)$ will denote the supersaturation at the time precipitation occurs. The expression applies approximately only when Q is large compared with 5. The degree of supersaturation is well illustrated for the precipitation of barium sulphate from solutions of barium thiocyanate and manganese sulphate, respectively. When the concentrations of these solutions were greater than molar, a gelatinous precipitate was instantly obtained, whereas with very dilute solutions ($< 10^{-3}$ M) the precipitate appeared after about one month and contained relatively large crystal particles.

This result indicated that the particle size of precipitate decreases with increasing concentration of the reactants. For the production of a crystalline precipitate, for which the adsorption errors will be least and filtration will be easiest, $(Q–S)/S$ should be as small as possible. There is obviously a practical limit to reducing $(0 - S)/S$ by making Q very small, since for a precipitation to

be of value in analysis, it must be complete in a comparatively short time and the volume of Solutions involved must not be too large.

In gravimetric analysis, the following applications are to be found :

1. Precipitation is carried out in hot solutions, since the solubility generally increases with rise of temperature.

2. Precipitation is effected in dilute solution and the reagent is added slowly and with thorough stirring. The slow addition results in the first particles precipitated acting as nuclei which grow as further material precipitates.

3. A procedure which is commonly employed to prevent supersaturation form Occurring is that of precipitation from homogeneous solution. This is achieved by generating the precipitating agent within the solution by means of a homogeneous reaction at a similar rate to that required for precipitation of the species.

The Purity of the Precipitate : Co-Precipitation

When a precipitate separates from a solution, it is not always perfectly pure; it may contain impurities depending upon the nature of the precipitate and the conditions of precipitation. The contamination of the precipitate by substances which are normally soluble in the mother liquor is termed co-precipitation. There are two important types of co-precipitation. The first is concerned with adsorption al the surface of the particles exposed to the solution, and the second relates to the occlusion of foreign substances during the process of crystal growth from the primary particles.

With regard to surface adsorption, this will be greatest for gelatinous precipitates and least for those of macrocrystalline character. Precipitates with ionic lattices appear to conform to the Paneth-Fajans-Hahn adsorption rule, which states that the ion that is most strongly adsorbed by an ionic substance (crystal lattice) is that ion which forms the least soluble salt. Thus on sparingly soluble sulphates, it is foung that calcium ions are adsorbed preferentially over magnesium ions because calcium sulphate is less soluble than magnesium sulphate.

The second type of co-precipitation may be visualised as occurring during the building up of the precipitate from the primary particles. The latter will be subject to a certain amount of surface adsorption, and during their coalescence the impurities will either be partially eliminated if large single crystals are formed and the process takes place slowly, or, if coalescence is rapid, large crystals composed of loosely bound small crystals may be produced and some of the impurities may be entrained within the walls of the large crystals. if the impurity is isomorphous or forms a solid solution with the precipitate, the amount of co-precipitation may be very large, since there will be no tendency for elimination during the 'ageing' process. The latter actually occurs during the precipitation of barium sulphate in the presence of alkali nitrates. In this particular case X-ray studies have shown that the abnormally large co-precipitation is due to the formation of solid solutions.

POST PRECIPITATION

Appreciable errors may also be introduced by post-precipitation. This is th

precipitation which occurs on the surface of the first precipitate after its formation It occurs with sparingly soluble substances which form supersaturated solutions; they usually have an ion in common with the primary precipitate. Thus in the precipitation of calcium as oxalate in the presence of magnesium, magnesium oxalate separated out gradually upon the calcium oxalate; the longer the precipitate is allowed to stand in contact with the solution, the greater is the error due to this cause. Post-prepitation differs from co-precipitation in several ways :

(a) The contamination increases with the time that the precipitate is left contact with the mother liquor in post-precipitation, but decreases co-precipitation.

(b) With post-precipitation, contamination increases faster agitation of solution by either mechanical or thermal means. The reverse is usually! with co-precipitation.

(c) The magnitude of contamination by post-precipitation may be much greater than in co-precipitation.

DIGESTION

This is carried out by allowing the precipitate to stand for 12-24 hours at room temperature, or sometimes by warming the precipitate for some time in contact with the liquid from which it was formed. The object is to obtain complete precipitation in a form which can be readily filtered. During the process of digestion or of the ageing of precipitates, at least two changes occur. The very small particles, which have greater solubility than the larger ones, will, after precipitation has occurred, pass into solution, and will ultimately re-deposit on the larger particles, co-precipitation on the minute particles is thus eliminated and the total co-precipitation on the ultimate precipitate is reduced. The rapidly formed crystals are of irregular shape become more regular in character and also more dense, thus resulting in decrease in the area of the surface and a reduction of adsorption. The net result of digestion is to reduce the extent of co-precipitation and to increase the size of the particles, causing filtration easier.

CONDITIONS OF PRECIPITATION

A number of general rules may be discussed to all cases of precipitation :

1. Precipitation should be carried out in dilute solution, due regard being paid to the solubility of the precipitate, the time required for filtration, and the subsequent operations to be carried out with the filtrate. This will minimise the errors due to co-precipitation.

2. The reagents should be mixed slowly and with constant stirring. This will keep the degree of supersaturation small and will assist the growth of large crystals. In some cases order of mixing the reagents may be important. Precipitation may be effected under conditions which increase the solubility of the precipitate, thus further reducing the degree of supersaturation.

3. Precipitation is effected in hot solutions, provided the solubility and the Stability of the precipitate permit. Either one or both of the solutions should be heated to just below the boiling point. At the higher temperature : (a) the

solubility is increased with reduction in the degree of supersaturation, (b) coagulation is assisted and sol formation decreases, and (c) the velocity of crystallisation is increased.

4. Crystalline precipitates should be digested overnight, except in those cases where post-precipitation may occur. As a rule, digestion on the steam bath is desirable. This process decreases the effect of co-precipitation and gives more readily filterable precipitates. Digestion has little effect upon amorphous precipitate.

5. The precipitate should be washed with suitable dilute solution of a electrolyte. Pure water may cause peptisation.

6. If the precipitate is still appreciably contaminated as a result co-precipitation or other causes, the error may be reduced by dissolving it in a suitable solvent and then re-precipitating it.

PRECIPITATION FROM HOMOGENEOUS SOLUTION

The main objective of a precipitation reaction is the separation of a pure Solid phase in a compact and dense form which can be filtered easily. In the technique known as precipitation from homogeneous solution, the precipitant is not added such but is slowly produced by a homogeneous chemical reaction within the solution, By varying the rate of the chemical reaction producing the precipitant in homogeneous solution, it is possible to alter the physical appearance of the precipitate th, slower the reaction, the larger are the crystals formed.

Many different anions can be generated at a slow rate ; the nature of the anion is important in the formation of compact precipitates.

Hydroxides and basic salts : The necessity for careful control of the pH has long been recognised. The hydrolysis of urea, which decomposes into ammonia and carbon dioxide is used for this purpose :

$$CO(NH_2)_2 + H_2O = 2NH_3; + CO_2$$

Urea possesses negligible basic properties ($K_b = 1.5 \times 10^{-14}$), is soluble in water and its hydrolysis rate can be easily controlled. It hydrolyses rapidly at 90–100°C, and hydrolysis can be quickly reduced at a desired pH by cooling the reaction mixture. The use of a hydrolytic reagent alone does not result in the formation of a compact precipitate; the physical character of the precipitate will be very mud affected by the presence of certain anions. Thus in the precipitation of aluminium by the urea process, a dense precipitate is obtained in the presence of succinate, sulphate formate, oxalate, and benzoate ions, but not in the presence of chloride, chlorate perchlorate, nitrate, sulphate, chromate, and acetate ions. The preferred anion for the precipitation of aluminium is succinate. It would appear that the main function of t 'suitable anion' is the formation of a basic salt which seems responsible for (¥ production of a compact precipitate.

The urea method results in the deposition on the surface of the beaker of a this tenacious, and transparent film of the basic salt. This film cannot be removed by scraping with a 'policeman', It is dissolved by adding a few millilitre of hydrochloric acid, covering the beaker with a clock glass, and refluxing for 5-10 minutes.

The urea hydrolysis method may be applied also to :

1. the precipitation of barium as barium chromate in the presence ammonium acetate;

2. the precipitation of large amounts of nickel as the dimethylglyoximates and

3. the precipitation of aluminium as the oxinate.

Phosphates : Insoluble orthophosphate may be precipitated with phosphate ion derived from trimethyl or triethyl phosphate by stepwise hydrolysis. Thus, 1.8 M sulphuric acid containing zirconyl ions and trimethyl phosphate or heating gives a dense precipitate which is ignited and weighed as the diploysphosphate (pyrophosphate (ZrP_2O_7).

Metaphosphoric acid may also be used. it hydrolyses in warm acid solution forming phosphoric (V) acid. Thus bismuth may be precipitated as bismuth phosphate in a dense, crystalline form.

Oxalates : Urea may be used to raise the pH of an acid solution containing hydrogenoxalate ion $HC_2O_4^-$ for the slow generation of oxalate ion. Calcium oxalate may thus be precipitated in a dense form :

$$CO(NH_2)_2 + 2HC_2O_4^- + HO = 2NH_4^- + CO_2 + 2C_2O_7$$

Dimethyl oxalate and diethyl oxalate can also be hydrolysed to yield oxalate ion :

$$[C_2H_5)_2C_2O_4 + 2H_20 = 2C_2H_5OH + 2H^+ + C_2O_4^{2-}$$

Diethyl oxalate is generally preferred due to its slower rate of hydrolysis.

Calcium can be determined as the oxalate by precipitation from homogeneous. solution by cation release from the EDTA complex in the presence of oxatate ion.

Suiphates : Sulphate ion may be produced by the hydrolysis of aminosulphonic (sulphamic) acid.

$$NH_2SO_3H + H_2O = NH_4^+ + H^+ + C_2O_4^{2-}$$

The reaction is used to produce barium sulphate in crystalline form.

The hydrolysis of dimethyl! sulphate also provides a source of sulphate ion, and the reaction is used for the precipitation of barium, strontium, and calcium as well as lead.

$$(CH_3)_2\ SO_4 + 2H_2O = 2CH_3OH + 2H^+ + SO_4^-$$

WASHING THE PRECIPITATE

Most precipitates are produced in the presence of one or more soluble compounds, and it is the object of the washing process to remove these as completely as possible. Only surface impurities will be removed in this way. The composition of the wash solution will depend upon the solubility and chemical properties of the precipitate and upon its tendency of undergo peptisation, the impurities to be removed, and the influence of traces of the wash liquid upon the subsequent treatment of the precipitate before weighing. Hydrolysable substances will need the use of Solutions containing an

electrolyte which will depress hydrolysis. The wash liquid is used hot or at some other temperature will depend upon the solubility of the precipitate. If permissible, hot solutions are to be preferred because of the greatly solubility of the foreign substances and the increased speed of filtration.

It is convenient to divide wash solutions into three classes :

1. *Solutions which prevent the precipitate from becoming colloidal and passing through the filter.* The wash solution should contain an electrolyte The nature of the electrolyte is immaterial, provided it 1s without action upon the precipitate either during washing or during ignition. Ammonium salts are, therefore, widely used. Thus dilute ammonium nitrate solution's employed for washing iron (III) hydroxide [hydrated iron (III) oxide], and] per cent nitric acid for washing silver chloride.

2. *Solutions which reduce the solubility of the precipitate.* The wash solution may contain a moderate concentration of a compound with one ion in common with the precipitate. Most salts are insoluble in ethanol and similar solvents, so that organic solvents can sometimes be used for washing precipitates. Sometimes a mixture of an organic solvent (e.g. ethanol) and water or a dilute electrolyte is effective in reducing the solubility to negligible proportion.

3. *Solutions which prevent the hydrolysis of salts of weak acids and bases.* It the precipitate is a salt of a weak acid and is slightly soluble it may exhibit a tendency to hydrolyse, and the soluble product of hydrolysis will be 4 base. The wash liquid must, therefore, be basic. Thus $Mg(NH_4)PO_4$ may hydrolyse to give the hydrogen phosphate ion HPO} and hydroxide ion, and should be washed with dilute aqueous ammonia. If salts of weak bases. such as hydrated iron (III), chromium (II), or aluminium ion, are to be separated from a precipitate, e.g. silica, by washing with water, the salts may be hydrolysed and their insoluble basic salts or hydroxides may be generated with an acid :

$$[Fe(H_2O_6)]^{3+} \rightleftharpoons [Fe(OH)(H_2O)_5]^{2+} + H^+$$

The addition of an acid to the wash solution will prevent the hydrolysis of iron (HI) or similar salts : thus dilute hydrochloric acid will serve to remove iron (III) and aluminium salts from precipitates which are insoluble in this acid.

Solubility losses are reduced by employing the minimum quantity of wash solution for the removal of impurities. Washing is more efficiently carried out by the use of many small portions of liquid than with a few large portions, the total volume being the same in both cases. Under ideal conditions, where the foreign substance is simply mechanically associated with the particles of the precipitate, the following expression may be shown to hold :

$$x_n = x_0\left(\frac{u}{u+v}\right)^n$$

where x_0 is the concentration of impurity before washing, x_n, is the concentration of impurity after n washings, u is the volume in millilitres of the liquid remaining with the precipitate after draining, and v is the volume in millilitres of the solution used in each washing. From this expression it is important : (a) to allow the liquid ;o drain as far as possible to maintain u at a

minimum, and (b) to use a small volumes. of liquid and to increase the number of washings. Thus if u = 1 mL and v = 9 mL, five washings would reduce the surface impurity to 10^{-6} of its original value; one washing with the same volume of liquid i.e. 45 mL, would only reduce the concentration to 1/46 or 2.2 × 10 of its initial concentration.

In practice, the washing process is not quite so efficient as the above simple theory indicates, since the impurities are not only mechanically associated with the surface. Frequent qualitative tests must be made upon portions of the filtrate for some foreign ion which is known to be present in the original solution. As soon as these tests are negative, the washing is discontinued.

IGNITION OF THE PRECIPITATE : THERMOGRAVIMETRIC METHOD OF ANALYSIS

In addition to superficially adherent water, precipitates may contain :

(a) Adsorbed water, which is present on all solid surfaces in amount depending on the humidity of the atmosphere;

(b) Occluded water, present in solid solution or in cavities within crystals;

(c) Sorbed water, associated with substances having a large internal surface development, e.g. hydrous oxides; and

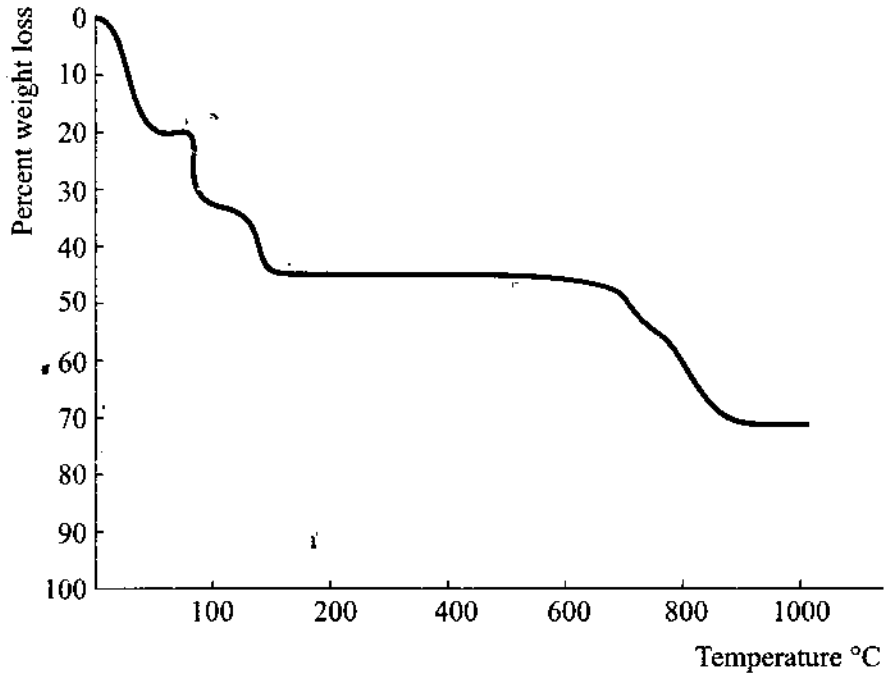
(d) Essential water, present as water of hydration or crystallisation [e.g., CaC_2O_4. H,O or $Mg(NH_4)PO_4$, $6H_2O$] or as water of constitution [water is not present as such but is formed on heating, e.g., $Ca(OH)_2 > CaO + H_2O$].

In addition to the evolution of water, the ignition of precipitates results in thermal decomposition reactions involving the dissociation of salts into acidic and basic components, e.g. the decomposition of carbonates and sulphates.

The temperature at which precipitates may be dried, or ignited to the required chemical form, can be determined from a study of the thermogravimetric curves for the individual substances. Thermogravimetric curves must be interpreted with Care, paying due attention to the different experimental conditions which apply in thermodygravimetry (temperature is usually changing at a regular rate) and in routine Rravimetric analysis (the precipitate is brought to a specified temperature and Maintained at that temperature for some definite time).

Thermogravimetry is a technique in which a change in the weight of a substance is recorded as a function of temperature or time. The basic instrumental requirement for thermogravimetry is a balance with a furnace programmed for a linear rise of temperature with time. The results can be represented (1) as a thermogravimetric (TG) curve, in which the weight change is recorded as a function of temperature or time, (2) as a derivative thermogravimetric (DTG) curve where the first derivative of the TG curve is plotted with respect to either temperature of time.

A Thermogravimetric curve, for copper sulphate ($CuSO_4$, $5H_2O$) is given below :



The following are the features of the TG curve :

(a) the horizontal protions indicate the regions where tehre is no weight change;

(b) the curve protions are indicative of weight losses;

(c) As the TG curve is quantitative, therefore, calculations on compound stoichiometry can be made at any given temperature.

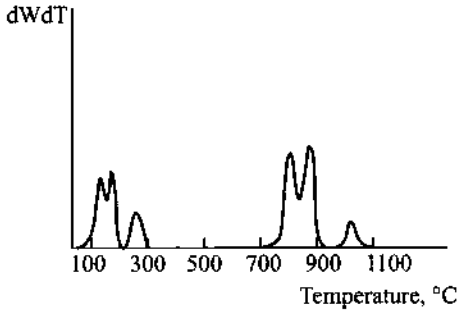
Copper sulphate pentahydrate shows four regions of decomposition as given below :

	Approximate temperature region
$CuSO_4.5H_2O \rightarrow CuSO_4.H_2O$	90–150°C
$CuSO_4.H_2O \rightarrow CuSO_4$	200–275°C
$CuSO_4 \rightarrow CuO + SO_2 + \frac{1}{2} O_2$	700–900°C
$2CuO \rightarrow Cu_2O + \frac{1}{2}O_2$	1000–1100°C

The precise temperature regions for each of the reactions are dependent upon the experimental conditions.

An additional features of the TG curve consists of the two region B and C with there are changes int he slope of the weight loss curve. If the rate of change of weight with time dW / dt is plotted against temperature, derivative thermogravimetric (DTG) curve is obtained. In the DTG curve when there is no weight loss the $dW = dt = 0$. The peak on the derivative curve corresponds to a maximum slope on the TG curve. When dW / dt is minimum but not zero there is an inflexion, *i.e.*, a change of slope on the TG curve. Inflexions B and C may imply the formation of intermediate compounds. In fact, the inflexion at B arises from the formation of the trihydrate $CuSO_4.3H_2O$ and that at point C due to formation of a golden-yellow basic sulphate of composition $2CuO.SO_3$. Derivative thermogravimetry is useful for many complicated determinations

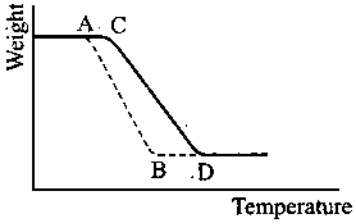
and any change in the rate of weight loss may be readily identified as a trough indicating consecutive reactions; hence weight changes occuring at close temperatures may be ascertained.



Experimental factors :

The precise temeprture regions for each reaction of the termal decomposition of copper sulphate pentahydrate are dependent upon experimental conditions.

The factors which may affect the results can be classified into two main groups of instrumental effects and the characteristics of the sample.



Instrumental factors : ***Heating rate :*** When a substance is heated at a fast rate the remperature of decomposition will be higher than that obtained at slower rate of heating. The effect is shown for a single-step reaction in the above curve. The curve AB represents the decompsotiion curve at a slwo heating rate, whereas the curve CD is due to faster heating rate. If T_A and T_C are the decomposition the final temperatures on completion of decomposition are T_B and T_D. The following features can be obtained :

$$T_A < T_C$$

$$T_B < T_D$$

$$T_B - T_A < T_D - T_C$$

The heating rate has only a small effect when a fast reversible reaction i considered. The points of inflexion B and C obtained on the thermogravimetric cur, for copper sulphate pentahydrate may be resolved into a slope, if a slower heating ra, is used. Hence the detection of intermediate compounds by thermogravimetry dependent upon the heating rate employed.

Furnace atmosphere : The nature of the surrounding atmosphere can have, profound effect upon the temperature of a decomposition stage. For example, th decomposition of calctum carbonate occurs at a much higher temperature if carboy dioxide rather than nitrogen is employed as the

surrounding atmosphere. The function of the atmosphere is to remove the gaseous products evolved during thermogravimetry

The most common atmospheres employed in thermogravimetry are :

1. 'static air' (air from the surroundings flows through the furnace);

2. 'dynamic air', where compressed air from a cylinder is passed through the furnace at a measured flow rate;

3. nitrogen gas (oxygen-free) which provides an inert environment.

Atmosphere which takes part in the reaction : For example, humidified air has been used in the study of the decomposition of such compounds as hydrated metal salts.

As thermogravimetry is a dynamic technique, convection currents arising ina furnace wil] cause a continuous change in the gas atmosphere. The exact nature of this change depends upon the furnace characteristics so that differing thermogravimetric data may be obtained from different designs of thermobalance.

Crucible geometry : The geometry of the crucible can change the slope of the thermogravimetric curve. A flat, plate-shaped crucible is preferred to a 'high form' cone shape because the diffusion of any evolved gases is easier with the former type.

Sample characteristics : The weight, particle size, and the mode of preparation of a sample all govern the thermogravimetric results. A large sample can often create a deviation from linearity in the temperature rise. This is particularly true when a fast exothermic reaction is studied, for example, the evolution of carbon monoxide during the decomposition of calcium oxalate to calcium carbonate. A large volume of sample in a crucible can impede the diffusion of evolved gases through the bulk of the solid large crystals, especially in case of certain metallic nitrates which may undergo 'spitting' or 'spattering' when heated. Other samples may swell, or foam and even bubble.

Diverse thermogravimetric results can be obtained from samples, for example, TG and DTG curves show that magnesium hydroxide prepared by precipitation methods has a different temperature of decomposition from that for the naturally occurring material. It follows that the source and the method of formation of the sample should be ascertained.

Applications : The applications of thermogravimetry are such as :

1. The determination of the purity and thermal stability of both prim 3 secondary standards;

2. The investigation of correct drying temperatures and the suitability of various weighing forms for gravimetric analysis;

3, Direct application to analytical problems (automatic thermogravimetric analysis);

4. The determination of the composition of complex mixtures.

Thermogravimetry is a valuable technique for the assessment of the purity of materials. Many primary standards absorb appreciable amount of water when exposed to moist atmosphere. TG data can show the extent of this absorption and hence the most suitable drying temperature for a given reagent may be determined.

The most widespread application of thermogravimetry in analytical chemistry has been in the study of the recommended drying temperatures of gravimetric precipitates.

Thermogravimetry may be used to determine the composition of binary mixtures. If each component possesses a characteristic unique pyrolysis curve, then a resultant curve for the mixture will afford a basis for the determination of its composition. In such an automatic gravimetric determination the initial weight of the sample need not be known. A simple example is given by the automatic determination of a mixture of calcium and strontium as their carbonates.

Both carbonates decompose to their oxides with the evolution of carbon dioxide. The decomposition temperature for calcium carbonate is in the temperature range 650-850°C, whilst strontium carbonate decomposes between 950 and 1150°C. Hence the amount of calcium and strontium present in a mixture may be calculated from the weight losses due to the evolution of carbon dioxide at the lower and higher temperature ranges, respectively. This method could be extended to the analysis of a three-component mixture, as barium carbonate is reported to decompose at an even higher temperature (~ 1300°C) than strontium carbonate.

Thermogravimetric analysis has also been used in conjunction with other techniques, such as differential thermal analysis (DTA), gas chromatography, and mass spectrometry, for the study and characterisation of complex materials such as Clays, soils and polymers.

SELECTING AND HANDLING OF REAGENTS

The purest reagents should be used for quantitative analysis, the analytical reagent (AR) quality is generally employed. Certain manufacturers market chemicals of high purity, and each package of these analysed chemicals has a label giving the manufacturers limits of certain impurities. While handling the reagents following points shuld be kept in mind:

(1) Liquid reagents should be poured from the bottle, a pipette should never be inserted into the reagent bottle.

(2) Particular care should be taken to avoid contamination of the stopper of the reagent bottle. When a liquid is poured from a bottle, the stopper should never be placed on the shelf or on the working bench, it should be placed upon a clean watch glass. Stopper should be returned to the bottle immediately after the reagent has been removed.

(3) All reagent bottles should be kept scrupulously clean, particularly around of the neck or mouth of the bottle.

(4)

If there is any doubt about the purity of the reagents used, they should be tested by standard methods for the impurities, which might cause errors in the determination.

(5) Reagents which are used in analytical laboratory should be of Analytical Reagent grade. Select the best grade of chemical available for analytical work.

(6) Special grades of solvents, for special purpose should be used. For example, spectral grades or chromatographic grades.

(7) Whenever possible, pick the smallest bottle that will supply the desired quantity.

(8) Unless specifically directed, never return any excess reagent to a bottle. The money saved by returning excess is seldom worth the risk of contaminating the entire bottle.

(9) Unless directed otherwise, never insert spatulas, spoons or knives into a bottle that contains a solid chemical. Instead shake the capped bottle vigorously or tap it gently against a wooden table to break up encrustation, then pour out the desired quantity. If these measures are ineffective, in such cases a clean porcelain spoon should be used.

(10) Keep the reagent shelf and the laboratory balance clean and neat. Clean up spills immediately, even though some one is waiting to use the same chemical or reagent.

(11) Observe local regulations concerning the disposal of surplus reagents and solutions.

QUESTIONS

1. What is analytical chemistry ? Classify the analytical methods and describe the factors affecting the choice and selection of an analytical method.
2. What are the important points to remember before cleaning an analytical laboratory ?
3. What is the need for cleanliness and neatness of an analytical laboratory ?
4. Describe the safety measures of handling the reagents in an analytical laboratory.
5. What do you know by the term 'analytical balance' ? Give the types and explain about the single-pan balance.
6. Describe the principle and technology of electronic balance and the single-pan balance.
7. Write down the use, care, and types of analytical balances.
8. Write short notes on the following :
   (a) Laboratory note book
   (b) Analytical balance
   (c) Semi-micro and micro balance
   (d) Cleanliness and neatness in laboratory
   (e) Safety in the analytical laboratory
   (f) Role of analytical chemistry

(g) Selecting and handling of reagents

(h) Calibration of glasswares

9. Write a short essay on laboratory operations and techniques.
10. Write down the calibration and cleanliness of glasswares.
11. How will you prepare an analytical sample? Descrbe in brief about sample decomposition and dissolution.
12. What is an analytical balance? Give the errors in weighing an analytical sample.
13. How will you differentiate between :

    (a) Classical and Instrumental method

    (b) Electronic balance and single-pan balance

    (c) Parts per thousand and parts per million

    (d) Stoichiometric and non-stoichiometric

    (e) Normality and Formality

    (f) Molarity and Normality

    (g) Wet ashing and dry ashing
14. What do you know about sample decomposition? How will you decompose a sample by microwave heating ?
15. What are fluxes ? How do they help to decompose an analytical sample ?
16. Describe the method to decompose the material in an open vessel with inorganic acids.

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CHAPTER

2

ANALYSIS OF WATER

POLLUTION

The term pollution has been derived from the Latin word 'Pollutioen', meaning 'defilement from polluere', to soil or defile (make dirty). In recent times the word pollution is used to denote the contamination of water, soil or air.

The environmental pollution is the unfavourable alteration of our surroundings, completely or largely as a by-product of our actions, through direct or indirect effects of changes in energy pattern, radiation level, chemical and physical constitution and abundance of organisms.

Water is a universal solvent and renewable resource. These unique properties of water make it vulnerable to get polluted. Water can be regarded polluted when it changes its quality or composition either naturally or as a result of human activities, thus, becoming less suitable for drinking, domestic, agricultural, industrial, recreational, wild life and other uses for which it would have been otherwise suitable in its natural and unmodified form.

WATER POLLUTION

Water pollution can be defined in a number of ways, such as:

(1) "Water pollution means the presence of any substance in water which affects temporarily or permanently the quality of its usefulness."

(2) "The presence of any foreign material which is undesirable and objectionable–either solid, liquid or gas in water causes water pollution."

(3) "Water is polluted when its basic properties are changed and it becomes harmful for human health."

(4) "Any toxic material in water which changes either its chemical or physical properties causes water pollution."

(5) "Addition of any unwanted substance which changes the composition of water, smell, taste, colour, pH or dissolved oxygen causes water pollution."

(6) "Presence of radioactive substance in water which alters its basic properties gives rise to water pollution."

(7) "Any unusual activities of man, which make water unfit for all living beings, directly or indirectly, cause water pollution."

(8) "Reduction of oxygen, addition of pathogens, and increase of toxicity in water which may alter the physical or biological properties of water are responsible for water pollution."

WATER POLLUTANTS

A water pollutant can be defined as a physical, chemical or biological factor causing aesthetic changes or making it detrimental to aquatic life and those who consume water. Majority of water pollutants are:

(1) In the form of chemicals: Which remain dissolved or suspended in water and give an environmental response, which is not acceptable.

(2) Physical Factors: Among the physical pollutants, heat and radiation are important which have a marked effect on organisms.

(3) Biological Factors: Certain microorganisms present in water, specially pathogenic species, cause diseases of man and animals and can be referred to as bio-pollutants.

Classification of Pollutants

Pollutants can be classified into following groups:

1. Common ions, salts or fixed dissolved solids.
2. Heavy metals and inorganic toxicants.
3. Organic material or BOD (biological oxygen demand).
4. Coliforms and other bacteria and viruses.
5. Nutrients particularly N, P, K.
6. Pesticides, herbicides and other toxicants.

1. Common ions, salts or fixed dissolved solids : These include the non-biodegradable salts which are quite soluble in water, and are responsible for the high electrical conductance *e.g.*, chlorides, sulphates, bicarbonates and nitrates of sodium, potassium, magnesium and calcium.

2. Heavy metals and inorganic toxicants : The concentration of these is generally low in water. Even when large amounts of these are added from industrial sources, these are quickly removed from the flowing water through precipitation, bioflocculation and settling on to the bed/bottom.

3. Organic material or BOD : Pollutional loads of organic matter or BOD are associated with discharge of nutrients. Some industrial processes discharge a lot of inorganic matter. These industries are ammonia, urea or phosphoric acid plants, distilleries, tanneries, pulp and paper factories, petrochemicals and rubber factories etc.

4. Coliform and other bacteria–viruses : This type of pollution comes only from human and animal wastes, and agricultural and industrial waste water. Coliform counts in Indian water bodies are always very high, often above $\frac{10^4}{100}$ mL.

5. Nutrients : Nutrients as pollutants, are found in all domestic and agricultural waste waters. Several industrial processes add large amounts of nutrients. Many rural and small industries e.g., rice-mills, flour-mills, pulse-mills, gur and khandsari units add significant amounts of N, P, K and S in water.

6. Pesticides, herbicides and other toxicants : Agricultural residues are largest sources of pesticides and herbicides, which are found in water at low concentrations.

ORIGIN OF WASTE WATER

The origin of waste water can be traced to its natural occurrence on the earth, formation by transformation and concentration of natural substances and their man-made synthesis.

Waste water is generated by moving the pollutants from domestic, industrial, agricultural use. The generation of sewage and waste water containing agrochemicals, certain pesticides and surfactants, petrochemicals, hydrocarbons, heavy metals and radionuclides are some important examples which are responsible for the origin of waste water.

Many chemicals do not occur in nature and pollution caused by them is entirely man-made. For example, synthesis of various pesticides, surfactants, radionuclide, and petrochemicals has introduced a large number of chemicals in the environment that has created severe environmental problems and has generated waste-water. Several of these compounds like plastics and some pesticides are nonbiodegradable resulting in a continuous build-up of their concentration in the environment. Some pollutants move from one component to another (*i.e.*, air = water, air = soil, soil = water) finally contaminating the whole environment.

EFFECTS OF WATER POLLUTANTS

Effects of water pollution can be classified into following categories:

1. Physico-chemical Effects
2. Biological Effects
3. Toxic Effects
4. Pathogenic Effects

1. Physico-chemical Effects : A large number of pollutants impart colour, taste and odour to the recipient water, thus, making it unaesthetic and even unfit for domestic consumption. The changes in oxygen content, temperature and pH value affect the chemistry of water, often triggering chemical reactions resulting in the formation of unwanted by-products.

The addition of organic matter results in depletion of oxygen with concomitant increase in carbon dioxide owing to bacterial degradation. The direct addition of nutrients through various sources enhances the algal and other biological growths which when die and decompose, they, thus further deplete the oxygen. The decomposition of excessive organic matter, when undergoes in absence of oxygen, results in odorous and unaesthetic conditions due to accumulation of several obnoxious gases like ammonia, hydrogen sulphide and methane, and several other intermediate substances.

The algal photosynthesis, when consumes carbondioxide, increases pH of water due to formation of carbonates which get precipitated as calcium carbonate often co-precipitating phosphorus with it. However, pH can fall at the time of higher rate of organic matter decomposition and low photosynthetic activity which can bring back the precipitated calcium carbonate in solution from the sediments.

2. Biological Effects : The addition of Pollutant leads to the shift in flora and fauna due to homeostatic factors operating in the aquatic system. Most of the fresh water algae are highly sensitive to pollutants and their elimination modifies the prey-predatory relationships by breaking down the food chain. This results in change of the whole plant and animal communities. The varieties of organisms decrease leaving presence of only a few tolerant forms in the polluted conditions.

The first response to the added nutrients is increased algal growth which is often composed of obnoxious bloom forming blue-green, or green chlorophycean algal forms. Many of the blue-green are not consumed by predators and some even produce toxic secretion causing allelophatic effects (*e.g.*, Microcystic spp). In the long run the ecosystem may change from autotrophic to heterotrophic. The accumulation of organic matter increases the density of saprophytic organisms. The game fish are replaced by hardy and inedible forms.

3. Toxic Effects : These are caused by pollutants such as heavy metals, biocides, cyanide and other organic and inorganic compounds which are

detrimental to organisms. These substances usually can have very low permissible limits in water, and their presence beyond these limits can render the water unfit for aquatic biota and even for human use. These chemicals (heavy metals, pesticides, PCBs, PAEs, PAHs, dioxins and surfactants) are toxic to the aquatic organisms, and many of them, specially those non-biodegradable, accumulate in the body of organisms and bio-magnify along the trophic lavels causing long lasting effects.

4. Pathogenic Effects : Besides the chemical substances, a few wastes like sewage, also contain several pathogenic and nonpathogenic microorganisms and viruses. The Clostridium perfringens and Streptococcus faecalis cause various types of food poisoning. Apart from this, many water borne diseases like cholera, typhoid, paratyphoid, colitis and infective hepatitis (jaundice) are spread by consumption of sewage contaminated water.

SOURCES OF WATER POLLUTION

The common sources of water pollution can range from purely natural to several man-made sources such as discharge of domestic and industrial waste in waters. Some common sources of water pollution may be described under the following categories :

1. Natural sources
2. Domestic sewage
3. Agricultural waste
4. Industrial waste
5. Radioactive waste.

1. Natural Sources : The natural entry of pollutants in water bodies can take place through rain, from atmosphere by dry deposition, entrapment and reaction, periodic submergence of surrounding vegetation, and falling of dry parts of nearby vegetation directly on the surface of water. The run-off water originated from different areas is quite rich in nutrients and organic matter. The run-off from sparsely populated or rural areas can pickup several substances from soil, including nutrients and organic debris. The run-off originating from the urban areas can collect vast quantities of substances.

2. Domestic Sewage : Sewage consists of water-borne wastes of the community, and contains about 99% water and 1% soil. Of the organic constituents, 65% are proteins, 25% are carbohydrates, and 10% fats. The inorganic fraction of sewage constitutes grit, salts and metals in varying proportions. The major problems associated with sewage are the production of odours and spread of enteric diseases, besides organic pollution which leads to oxygen depletion and fish kill. Sewage also contains huge quantities of nutrients in the form of nitrogen and phosphorus even after the secondary treatment that often causes eutrophication. Another common way of sewage disposal is its land treatment or crop irrigation. However, its disposal in this way, without scientific considerations, can lead to the severe health hazards and deterioration of land in the long run.

3. Agricultural Wastes : Agricultural wastes usually originate in the form of run-off from the agricultural fields and animal farms. Modern agriculture uses a large number of chemicals, called agrochemicals, in the form of fertilizers, organic manure, pesticides, growth hormones, nutrient solutions and others. All residual forms of these chemicals along with organic debris from the remains of the harvested crops are trapped by run-off water, causing pollution problems in the receiving waters. The agricultural run-off water is considerably rich in nutrients like nitrogen and phosphorus, organic matter

and pesticides. While nutrients create the problem of eutrophication, pesticides have been reported to get bioaccumulated and bio–magnified through food chains resulting in the secondary poisoning to man and predatory birds.

4. Industrial Wastes : The industrial wastes have the greatest potential for polluting the recipient water. The nature and composition of industrial waste depends upon the raw materials, processes and operational factors. The industrial wastes may have pollutants of almost all kinds ranging from simple nutrients and organic matter to complex toxic substances. The wastes from the industries like sugar factories, dairies, paper and pulp industries, tanneries and distilleries are rich sources of organic matter. Metal plating industries release substantial quantities of heavy metals and cyanide in their wastes. The wastes from fertilizer industries can also be a rich source of nutrients causing eutrophication. The chemical industries release wastes with highly variable composition which are often acidic or alkaline in nature. The industrial wastes can be described in detail as follows :

(a) Pulp and Paper Industry : The chemicals used in the factory are : (1) Alum (2–3 tonnes), (2) Talc (0–10 t), (3) Rosin (1–1.25 t), (4) chlorine (1.5–3.5 t), caustic soda (150–250 kg), (6) Soda ash (150–180 kg), (7) dyes (2–50 kg), (8) Magnesium bisulphite, (9) Sulphurous acid, clay (2–15 t). The characteristics of a paper industry are as follows :

Characteristics of Pulp and Paper Industry

Parameters	Pulp unit		Bleaching operation unit	
	max	min	max	min
Temperature of effluent	39°C	28.2°C	32.8°C	27.8°C
Colour	Brownish	–	milky white	–
Turbidity	730	<85	>1000	140
Specific conductivity at 250°C $\times 10^{-6}$ mho	7.5	5.0	10.5	9.0
Total alkalinity as $CaCO_3$ (mg/mL)	1000	44	1134	84
D.O. (mg/litre)	2.9	0.0	0.0	0.0
B.O.D. at 20°C (mg/litre)	1920	400	133	84
Available chlorine (mg/litre)	–	–	1704	16

(b) Distillery: Annual distillery discharge in India is approximated between 100–110 million Litre and this can produce 10–250 tonnes nitrogen, 1000–2500 tonnes potash and 50–100 tonnes phosphorus besides aminoacids, nitrates and microorganisms like phytoplankton and zooplankton. The distillery wastes are highly organic in nature and because of their high biochemical oxygen demand (B.O.D.), quickly remove the oxygen from the water and unless wastes are diluted they can produce pollution hazards in the aquatic ecosystem. The diluted distillery effluent (1 : 200 dilution) gives birth to a number of green and blue algae.

Characteristics of Distillery Wastes

Parameters	Summer (March–June)	Rains (July–August)	Winter (Nov.–Feb.)
Temperature of water	38.5°-52.5°C	32°-42.1°C	38°-46°C
Turbidity (ppm)	385 > 1000	365 > 1000	1000 > 1000
pH	4.6–6.9	5.0–6.9	4.8–6.9
Colour	Brown–Deep brown	Brown-Deep brown	Brown–Deep brown
D.O. (ppm)	nil–nil	nil–0.2	nil–nil
COD (ppm)	3826–52000	654–11200	1080–27200
BOD ppm (20°C)	16000–2100	14100	5760–14850
Alkalinity (ppm)	308–4140	290–3600	490–9440
Sp. conductivity × 10^{-6} mho at 25°C	1028–14399	4235–13199	6041–8999

From Distillery Effluent

Green Algae	Blue–Green Algae
Chlorella, Scenedesmus, Chlorogonium	Oscillatoria, Phormidium, Anacystis, Anthrospria

(c) Potassic Fertilizer: The potassic ferlilizer contains 37% K_2O, rest being silica, Calcium, iron and some sodium is the end product of the process.

(d) Electroplating Plants: The electroplating plants contain harmful heavy metals and cyanides which cause excessive alkalinity or acidity in water, which is very harmful for aquatic life.

(e) Asbestos: Asbestos occurs in four forms in water as pollutant. These forms are: (i) Chrysolite, (ii) Crocidolite, (iii) Tremolite and (iv) Amosite. The size of asbestos particle in water is very small (less than 0.1 μ in diameter) hence it is not detected by light microscope. It can create cancer of intestine, lungs and stomach with respiratory tract.

(f) Silt: It is the component of soil, which has a diameter of 0.002 mm. Sandy loam soil and loam soil contains silt. The silt particles generally choke irrigation channels, rivers, and also disturb the aquatic life. Silt particles are carried from land to water and cause serious pollution of water resources.

(g) Alcohol Industry: In the fermentation of mollases besides alcohol, glycerine, succinic acid, acetaldehyde, allyl alcohol, acetic acid and fuse oil as by-products are formed, which cause water pollution.

(h) Detergents: The detergents cause serious water pollution as they contain phosphates. The phosphates are responsible for the growth of algae which deplete the dissolved oxygen. When algae die, they release their components, inorganic and organic substances, back into water and some pollutants themselves. Generally they give amino acids and ammonia in water.

(i) Steel Industry: From steel industry phenol (0.002 mg/l) and many other pollutants are released into water. Phenol gives unpleasant taste to water and chlorine, iron and manganese (if more than 0.1 mg/litre) give rise to bad taste in drinking water. The effluent is toxic to aquatic life as it contains high values of B.O.D. and C.O.D., phenol, cyanides and other constituents and a very low value of dissolved oxygen.

Characteristics of Steel Industry

Contaminants	Amount
pH	8.5–9.5 mg/L
Phenol (mohohydric, Polyhydric and derivatives of phenols)	500–100 mg/L
Total ammonia	800–1400 mg/L
Thiosulphate	110–220 mg/L
Thiocyante	50–100 mg/L
Chloride	4000–4200 mg/L
Cyanide	10–50 mg/L
Sulphide	10–20 mg/L
B.O.D. (5 days at 20°C)	160 mg/L
C.O.D.	790–2450 mg/L
Iron and manganese	more than 0.4 mg/L
Dissolved oxygen	2 mg/L

(j) Tanning Industry : Tanning Industry effluent gives the following constituents, which are responsible for water pollution:

Characteristics of Tanning Industry

Constituents	Amount
pH	5.5–9.0
Hexavalent Chromium	0.1 mg/L
B.O.D. (5 days at 20°C)	30 mg/L
Chloride	1000 mg/L

(k) Cane sugar Industry : Generally the sugar mills are in rural areas where effluents pollute small rivers and give foul smell in the surroundings of the mill. As the effluent contains a high degree of organic pollution hence within few hours, biological action starts and septic conditions give H_2S gas imparting black colour to the effluent. Thus oxygen is also exhausted, causing death of fish and other aquatic life. This water is extremely harmful to the plants. The following are the characteristics of the effluents:

Characteristics of Cane sugar Industry

Characteristics	Amount
Total Solids	870–3500 mg/L
Suspended Solids	220–800 mg/L
Volatile Solids	400–2200 mg/L
pH	4.6–7.1 mg/L
B.O.D. (5 days at 20°C)	300–2000 mg/L
C.O.D.	500–4380 mg/L
Total nitrogen	10–40 mg/L
Colour	light Brown

(l) Oils: Oils are toxic substances too which affect the living organisms in water.

(m) Pesticides and Herbicides : The pesticide and herbicide compounds, when sprayed on plants to kill harmful pests and weeds, percolate through soil and get dissolved in soil water thus polluting it. The compounds D.D.T., B.H.C., endrin, heptachlor, and toxaphene are also washed down with rain water and find their way to sea through rivers and streams. The compounds accumulate in the bodies of aquatic animals and plants. Some amount of pesticides is also found in the milk of cow etc., in the vegetables, and in eggs besides other edible things.

The modern pesticide consists of 5–following groups:

(i) Chlorohydro–carbons (*e.g.*, D.D.T., Aldrin, Endrin etc.)

(ii) Carbamates (*e.g.*, Sevin)

(iii) Organo-phosphorus compounds (*e.g.*, parathion, malathion)

(iv) Inorganic pesticides include compounds of lead, arsenic, mercury, chlorine, HCN, lead arsenate, and sodium arsenate.

(v) Naturally occurring pesticides are rolenone, nicotine, and petroleum derivatives.

Characteristics of Pesticide Industry

Characteristics	Maximum	Minimum
pH	2.0	0.5
B.O.D. (5 days at 20°C)	890 mg/L	nil
C.O.D. (dichromate method)	8330 mg/L	1040 mg/L
Total Nitrogen as N	4.20 mg/L	0.45 mg/L
Chromium as Cr	4.75 mg/L	0.45 mg/L
Chloral hydrate	4300 mg/L	255 mg/L
Acidity as $CaCO_3$	75740 mg/L	640 mg/L
Total dissolved solids	10000 mg/L	15000 mg/L

(n) Drug Industry or Organic Chemical Industry: The Industry can be classified into 3 groups:

(i) Antibiotics, (ii) Natural drugs and (iii) Synthetic drugs

The composition of waste water from synthetic drug factory producing anti- byretics, sulpha drugs, antitubercular drugs and vitamins and main characteristics are given below:

Characteristics of a drug Industry

Characteristics	Range
pH	0.8
Total solids	8.6%
Total volatile solids	5%
Chloride as Cl	18500 mg/L
Sulphates as SO_4	23000 mg/L
Total Nitrogen as N	6100 mg/L
Phosphates as P	nil
COD	19700 mg/L
BOD	13000 mg/L
Mineral acidity as $CaCO_3$	28000 mg/L

(5) Radioactive Wastes: The radioactive substances are used for power industry, heating homes, preserving food, fuelling, transport, and as medicines for curing diseases. They can also be used to prepare bombs etc.

The wastes from atomic reactors, hospitals, etc. are most dangerous because their radioactivity can not be destroyed easily at human will. These wastes destroy the aquatic plants and animals to a great extent. They generally cause gene mutation, ionization of body fluids and chromosomal mutation. The requirements for discharging radioactive waste water into the biological stream are given below.

Requirements for discharging waste water into the biological stream

Constituents	Effluent standard
pH	5.5–9.0
Suspended solids	100 mg/L
Oil and grease	10 mg/L
Temperature	40°C
Radioactive substances:α-particles	10^{-7} μ/mL
β-particles	10^{-8}μ/mL
Phenols	1.0 mg/L
Sulphides	2.0 mg/L
BOD concentration of average	30 mg/L
COD	250 mg/L
Ammonical nitrogen	50 mg/L
Total residual chlorine	1 mg/L
Arsenic	0.2 mg/L
Lead	0.1 mg/L
Nickel	3.0 mg/L
Selenium	0.05 mg/L
Zinc	5 mg/L
Mercury	0.01 mg/L
Copper	3.0 mg/L
Cadmium	2.0 mg/L
Fluoride	2.0 mg/L

TYPES OF WATER POLLUTION

Water pollution can be divided into eight categories, as given below:

1. Physical Pollution
2. Inorganic Pollution
3. Organic Pollution
4. Biological Pollution
5. Oil Pollution
6. Garbage Pollution
7. Pesticide Pollution
8. Radioactive Pollution

1. Physical Pollution : This type of pollution changes the physical properties of water. For example, if colour is added to water, and it becomes coloured, the water can be said to be polluted. The acceptable value of colour is 5.0 on platinum cobalt scale. Similarly, any change in odour or taste of water means it is polluted due to some pollutants, even if its chemical properties remain the same. Sometimes colloidal particles from industries and other sources along with clay, change the turbidity of water and make it polluted. The maximum limit of turbidity in drinking water is 10 J.T.U scale. If the value of turbidity is higher than the prescribed limit, it can be removed by treating the water with alum or some other methods (Fig. 17).

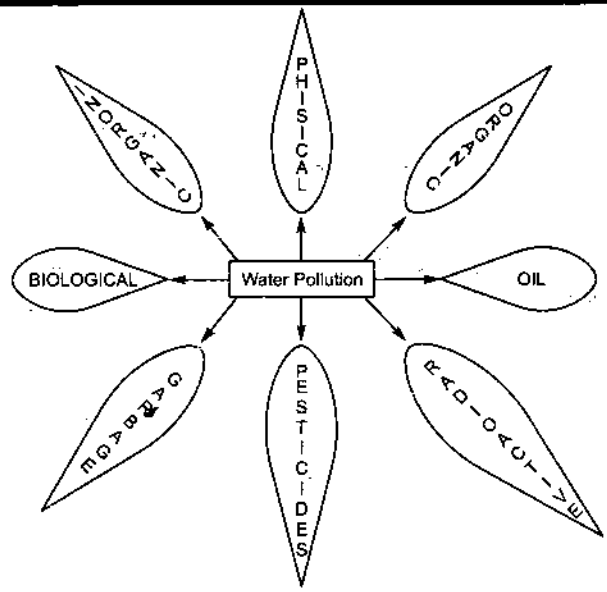


Fig. 17 Types of Water Pollution

2. Inorganic Pollution: There are many industrial units which discharge sulphides, nitrites, sulphates, phosphates etc. in river water making it unfit for drinking. These inorganic substances decompose slowly and slowly in water, releasing foul gases and produce several by-products which change the basic properties of water and also its pH.

The reduction of pH to 4 or 5 makes water acidic and becomes responsible for the killing of aquatic life. The acceptable pH of drinking water is 7–8.5. The nitrites and nitrates are responsible for a number of diseases in human beings and even death of infants. The inorganic pollutants change chemical oxygen demand of water and thus make it unfit for human consumption.

3. Organic Pollution: The organic compounds which are discharged from industries and other sources change pH of water drastically and also change dissolved oxygen, biochemical oxygen demand and produce several by-products which pollute water.

The pesticides, fungicides, bacteriacides etc. are organic substances which are mainly used for killing small insects etc; but they persist in nature for long time and due to their biodegradable nature, they create serious water pollution complications and enhance B.O.D. to the order of 30,000 mg/L, while in drinking water this value should be zero. Thus these non-biodegradable substances make water unfit for human consumption permanently.

The bio-degradable substances such as proteins, oils, carbohydrates, starch, sugars, fats, food materials etc. also pollute water, although their effects are of temporary nature.

4. Biological Pollution: This type of pollution is caused by plant toxins, coliform bacteria, sub groups faecal streptococci, multiorganisms, algae, viruses, rotifers etc. The micro-organisms and viruses are responsible for various water diseases like cholera, typhoid, dysentery, hepatitis, gastroenteritis, polio etc. Birds of different genera also pollute drinking water.

5. Oil Pollution: The pollution in water by oil is called oil pollution. When oil is transported from one place to another or from one country to another through sea, oil tankers either explode due to enemy's attack or due to leakage in ports or docks. The oil is made up of about 40 components. The upper components like petrol and kerosene evaporate causing air pollution in

nearby area, while lower components like nephtha etc. destroy the plant and animal life of the sea, being thicker in nature thus neither allowing sun light to enter into the lower portion of sea nor allowing oxygen. Thus oil in water is responsible for:

(a) Reducing light penetration : Oil-slick forms a layer upto 2 meters below the surface of water, which checks light from reaching the lower part of sea, needed for photosynthesis of aquatic plants.

(b) Reducing dissolved oxygen : Oils reduce the percentage of dissolved oxygen in sea with the result that aquatic animals die due to lack of oxygen necessary for their respiration. The maximum permissible limit of oil in water is 0.3 mg/L. Any higher amount than this prescribed value causes oil pollution. The oil pollution can be controlled by checking spillage of oil.

EFFECTS OF OIL POLLUTION

(i) Crude oil contains small amount of saturated hydrocarbons, nitrogen, sulphur compounds, metals like iron, nickel, vanadium etc. which cause paralysis.

(ii) Liquid paraffins can cause pneumonia in lungs and can destroy the tissues of kidney. Aromatic compounds like mercaptans, thiophene and benzothiophene are toxic in nature and damage kidney.

(iii) The saturated hydrocarbons like methane, ethane, propane etc. cause suffocation and respiration ailments.

(iv) The oily furs of animals too lose insulation resulting in death finally.

(v) Crude oil contains carbonyl sulphides also which breaks up into hydrogen sulphide, a poisonous gas which affects the respiratory system.

6. Garbage Pollution: Garbage is the major source of pollution of rivers like the Ganga, Gomti, Yamuna, Godavari, Krishna, Kaveri, Mahanadi, Damodar, Sone etc. as the garbage is thrown into the rivers by villagers without any treatment. The stagnant puddles of water, human and animal excreta have become a common part of the neighbourhood scene in the cities, and has become centre of pathogens infections and breeding ground for carriers of plague, mosquitoes and other communicable diseases. The high concentration of various toxic elements may also create problems for ponds, rivers and other water bodies, which are sources of drinking water for human beings.

7. Pesticides Pollution: For the last 60 years pesticides are being transmitted through water in the environment. Pesticides also find way into overground water bodies thus affecting aquatic life and polluting water for a long time or for ever. Poultry also gets affected by pesticides. Domestic animals like cows, pets etc. and human beings ingest the remains of pesticides through fodder and food respectively. Eggs, milk, curd, ghee, meat products etc. are all found affected by pesticides. The human beings thus contact new diseases. Many of vegetables like potatoes, onions, brinjals, cauliflower etc. also contain small quantities of pesticides absorbed through soil. These pesticides are also found in food grains, wheat, rice, maize, bajra etc. and sometimes cause failure of liver gonads and kidney functioning in human beings.

8. Radioactive Pollution: Radioactive pollution is caused by the use of radioactive material through the activities of man. Among radioactive substances, radionuclides are heavily toxic in water. The radioactive

substances enter into environment and finally mix in rivers and other water bodies and contaminate them. The following are the major sources of radioactive substances:

(a) Through mining and processing of ores.

(b) Through production of isotopes.

(c) Through nuclear power plants installed for generation of electricity.

(d) Through nuclear reactors.

(e) Through leakage of nuclear detonators.

(f) Through explosion of nuclear bombs and weapons.

The radioactive substances in water may cause DNA breakage, eye cataract, leukemia and blood cancer. The radioactive substances in water react with proteins of animals and deactivate enzymes by breaking S—H—S linkage of bonds, causing stoppage of cell division at once. The radiation splits the water into H^+ and OH^- and H^* and OH^* free radicals. These free radicals react with cells and damage them completely in human and animals both. The Rn–226, Rn–228, Rn–232 in drinking water cause stomach disorders, birth abnormalities, genetic change etc. Sr–90 and Fe–65 in water cause death of fish and break the food chain and metabolic activities. Testing of bombs and waste materials of labs etc. are continuously posing threat to human life causing skin cancer, deformity in bones, loss of hair, nausea, haemorrhagic diseases, congestion in throat and bones necrosis.

WATER ANALYSIS

COLOUR

Pure water has no colour. The presence of humic acids, fulvic acids, metallic ions, suspended matter, phytoplanktons, weeds and industrial effluents may give colour to natural water. Colour can be determined by following two methods:

1. Platinum Cobalt Method
2. Forel Ule Colour Scale Method

1. Platinum Cobalt Method: This method gives a quantitative measure of the dominant spectral colour (hue).

Materials

1. Nessler Tubes: 50 mL

2. Colour Standards: Dissolve 1.0 g of crystalline cobaltous chloride and 1.245 g of potassium chloroplatinate in a small quantity of distilled water. Add 100 mL of concentrated hydrochloric acid and dilute to 1 litre. This solution has a colour value of 500 colour units. Prepare standards by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, mL of above solution with distilled water to 50 mL in standard Nessler tubes. These solutions have colour value of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70 respectively. Indicate the colour value on each tube and protect them from evaporation and contamination.

Method

1. Centrifuge the sample at high speed to remove suspended matter.

2. Fill standard Nessler tube with sample to the same level as that of standard (50 mL).

3. Compare the colour of sample with that of various standard tubes held

vertically above a white surface and find one standard with same colour (hue) as that of sample and read its colour value.

Note: 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.

2. Forel-ule Colour Scale Method: This method gives a qualitative recognition of the colour of sample.

Materials

1. Solution S–1: Add 0.5 g of $CuSO_4 \cdot 5H_2O$ to 5 mL strong NH_4OH and dilute to 100 mL with distilled water.

2. Solution S–2: Add 0.5 g of $K_2CrO_4 \cdot 5H_2O$ to 5 mL of strong NH_4OH and diltue to 100 mL with distilled water.

3. Solution S–3: Add 5g of $CaSO_4 \cdot 7H_2O$ to 5 mL of strong NH_4OH and dilute to 100 mL with distilled water.

Empirical Forel-Ule colour scale is prepared by mixing different proportions of the above solutions (solution I, II, III).

Method

1. Centrifuge the sample so as to remove the suspended matter.

2. Fill the sample in a clean tube and compare its colour with 22 mixtures of the Forel-Ule colour scale.

3. Find matching colour and note the colour of sample from this scale.

Forel–ule Colour Scale

Solution	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
S–1 (mL)	100	98	95	91	86	80	73	65	56	46	35	35	35	35
S–2 (mL)	0	2	5	9	14	20	27	35	44	54	65	60	55	50
S–3 (mL)	0	0	0	0	0	0	0	0	0	0	0	5	10	15
	Blue		Greenish blue		Bluish green			Green			Greenish yellow			

Solution	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII
S–1 (mL)	35	35	35	35	35	35	35	35
S–2 (mL)	45	40	35	30	25	20	15	10
S–3 (mL)	20	25	30	35	40	45	50	65
	Greenish yellow			Yellow brown			Brown	

TURBIDITY

Turbidity in water is caused by suspended matter like clay, silt, organic matter, phytoplanktons and other microscopic organisms. It is an expression of optical property (Tyndall effect) in which light is scattered by the suspended particles present in water. Scattering of light is dependent upon the size, shape and refractive index of such particles.

Turbidity when caused (i) Largely because of phytoplankton, is considered as an index of productivity, but on the contrary, when caused (ii) because of suspended matter other than phytoplankton, it restricts the light penetration in water resulting in reduced primary production (photosynthesis).

Material

1. Nephelometer (Turbiditymeter)

2. Sample Tubes: Made of coloured and scratch-free glass.

3. Standard Turbidity Suspension : Dissolve 1 g of hydrazine sulphate in distilled water to prepare 100 mL of solution (a) Dissolve 10 g of hexamethylene tetraamine in distilled water to prepare 100 mL of solution (b) Mix 5 mL of each of the solutions (a and b) in 100 mL volumetric flask and allow to stand for 24 hours at about 25°C. Dilute it with distilled water to the mark. This is a suspension having 400 NTU (Nephelometric Turbidity Unit), and can be stored for about one month.

Dilute 10 mL of above stock solution (of 400 NTU) to 100 mL with distilled water. This standard solution has 40 NTU and can be stored for a week.

Method

1. Set the nephelometer at 100 using 40 NTU standard suspension. In doing so every percent of the scale will be equal to 0.4 NTU turbidity.

2. Shake the sample thoroughly and let the air bubbles subside.

3. Take the sample in nephelometer sample tube and find out the value on scale. If the sample has turbidity more than 40 NTU, dilute it, so that its turbidity can be read on the same scale.

Calculation : Turbidity (NTU) = Nephelometer reading × 0.4 × dilution factor

TOTAL DISSOLVED SOLIDS

Salts like carbonates, bicarbonates, chlorides, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron and manganese etc. are dissolved in natural water. The high content of dissolved solids increases the density of water and influences osmoregulation of fresh water organisms. They reduce solubility of gases (like oxygen) and utility of water for drinking, irrigation and industrial purposes.

Materials

1. Evaporating dish
2. Chemical balance
3. Desiccator
4. Hot water bath
5. Filter paper (Whatman No. 4)

Method

1. Filter 250 to 500 mL of sample through Whatman No. 4 filter paper in a pre-weighed evaporating dish.

2. Evaporate the sample on hot water bath until whole water is evaporated.

3. Note the weight of evaporating dish after cooling it in a desiccator and calculate total dissolved solids.

$$\textbf{Calculation :}\ \text{TDS} = \frac{(\text{Weight of dry solid + dish}) - \text{Weight of dish}}{\text{Volume of sample taken (mL)}} \times 100$$

where,

TDS = Total dissolved solids (g/L); all the weights are taken in gram

TOTAL SOLIDS

Materials and Method: For total solids, do not filter the sample through whatman No. 4 filter paper. Rest of the materials and method are same as given in total dissolved solids.

Calculation: Use the same formula as given in total dissolved solids.

CONDUCTIVITY

The cell of the conductivity meter or solubridge is filled with water sample and the electrical conductivity (*EC*) is measured. It is expressed as ds/m at 25°C which is numerically equal to m mhos/cm at 25°C. Sometimes the unit of expression is milli–Simons/cm (ms/cm) which is numerically the same as micromhos/cm that is 1/100th of deci Simons per metre or millimhos/cm.

Soluble salts present in water dissociate into their respective cations and anions. These cations and anions carry current and impart conductivity. Higher the concentration of ions, greater is its electrical conductance (less the resistance to electric current). Thus the measurement of *EC* is directly related to the soluble salt concentration. Although, the relationship between conductivity and salt concentration varies somewhat depending on the ionic composition of the solution, the electrical conductivity provides a rapid and reasonably accurate estimate of solute concentration. Conductance is the reciprocal of the resistance involved and the unit of measure of conductance is reciprocal of ohm designated as mhos or Siemens (S). When electrical conductivity is measured between the electrodes having a surface area of $1\,cm^2$ and placed at a distance of 1 cm, the unit of conductivity is S/cm.

The *EC* is directly proportional to the area (surface area) and inversely proportional to the length (distance). The same is expressed as:

EC (Electrical Conductivity)

or $$EC = K\, a/l$$

where, a = area, l = length,

K = Proportionality constant called specific conductance.

In case $a = 1$ sq. cm and $l = 1$ cm then conductivity = K, *i.e.*, specific conductance

Increase in temperature promotes dissociation with a consequent rise in conductivity, hence it is conveniently reported at 25°C.

Measurement of Electrical Conductivity

The instrument consists of an *AC* salt bridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black. The instrument is also available as an already calibrated assembly (solubridge) for giving the conductivity of the solutions in mili mhos per centimeter or deci Siemen per meter at 25°C.

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

$$\frac{r_1}{r_2} = \frac{r_3}{r_4}$$

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.

Equipment and Reagents

1. Conductivity meter and cell
2. Beaker

Standard Potassium Chloride solution (0.01M) : 0.7456 g of dry AR grade KCl is dissolved in freshly prepared double distilled water and made to one litre. At 25° C it gives an electrical conductivity of 1.413 in mhos/cm(dS/m). The instrument is to be checked and calibrated wth this solution.

Method

1. Take sample in 925 mL beaker.
2. Warm up the instrument for 20 minutes.
3. Use 0.01 M KCl solution to calibrate the meter.
4. Rinse the conductivity cell with distilled water and then with the sample.
5. Temperature and cell constant corrections are adjusted on the conductivity meter, it provided.
6. Connect the conductivity cell to meter and dip in the sample. Pass the current and adjust the current by rotating the dial in such a way that maximum sensitivity is obtained.
7. Read the conductivity value in dS/m. Direct reading may be obtained in digital type of meters.

Observed values of *EC* are multiplied by the cell constant (usually given on conductivity cell) and a temperature factor to express results at 25°C, if instruments are provided with temperature compensations in which reading directly comes at 25°C. Operating manual must be read before operation of the instrument.

ACIDITY

Sometimes water shows acidity due to presence of uncombined CO_2, salts of strong acids and weak bases and mineral acids. For determining the acidity of water, it is titrated with standard solution of strong base by using a suitable indicator.

Apparatus:

1. Conical Flask
2. Pipette
3. Burette

Reagents :

1. Phenolphthalein and methyl orange indicator
2. N/100 NaOH solution.

Procedure

1. Take 100 mL sample in a conical flask and add 2 drops of phenolphthalein indicator, and titrate it with N/100 NaOH solution.

2. A faint pink colour appearance is the end point of titration.

3. Note down the volume used of N/100 NaOH from burette.

Calculation : Total Acidity as $CaCO_3$ (mg/litre)

$$= \frac{\text{used volume of NaOH} \times N \times 100 \times 1000}{\text{Volume of sample taken (mL)}}$$

Where, N = Normally of NaOH solution.

ALKALINITY

Usually, water shows alkalinity due to presence of salts of weak acids, and strong bases. The alkalinity in water is caused due to presence of (a) Carbonates (CO_3^{--}), (b) Bicarbonates (HCO_3^-) and (c) Hydroxides (OH^-)

Alkalinity can easily be determined by titration using phenolphthalein (work in alkaline pH range above 8.2) or methyl orange indicator (work in acidic pH range below 6.0)

Reagents

1. Phenolphthalein Indicator: 0.25% solution in 60% ethyl alcohol.

2. Methyl orange Indicator: 0.5% solution in 95% alcohol.

3. Standard Sulphuric Acid (0.02N): Dilute 2.8 mL of concentrated H_2SO_4 to 1 litre with distilled water. Dilute 200 mL of this solution to again 1 litre for getting 0.02N H_2SO_4. Standardize this solution.

Method

1. In a porcelain dish 5 mL of the sample (containing not more than one milliequivalent of carbonate plus bicarbonate) is diluted with distilled water to about 25 mL.

2. Pink colour produced with a few drops (2–3) of phenolphthalein indicates presence of carbonates and it is titrated with 0.02 N sulphuric acid until the colour just disappears (phenolphthalein end point) because of alkali carbonate being converted to bicarbonate. This burette reading is designated as (A).

3. To the colourless solution from this titration (or to the original sample of water if there was no colour with phenolphthalein) 1–2 drops of methyl orange or methyl red indicator are added and the titration continued till the colour changes from yellow to rose red.

4. Record the final reading.

Suppose A = mL of acid used for titration with phenolphthalein.

B = mL of acid used for Total titration (with phenolphthalein and methyl orange both)

The observation can be summarised in the following ways:

(a) When $A = B$

Total alkalinity of hydroxide, mg/Litre $= \frac{A \times 1000}{\text{mL of sample}}$

(b) When $A > \frac{1}{2} B$

Total Alkalinity, (mg/litre) $= \frac{B \times 1000}{\text{mL of sample}}$

Hydroxide alkalinity, (mg/litre) $= \frac{(2A - B) \times 1000}{\text{mL of sample}}$

(c) When $A = \frac{1}{2} B$

$$\text{Total alkalinity, (mg/litre)} = \frac{B \times 1000}{\text{mL of sample}} = \text{Carbonate alkalinity}$$

(d) When $A < \frac{1}{2} B$

$$\text{Total alkalinity, (mg/litre)} = \frac{B \times 100}{\text{mL of sample}}$$

$$\text{Carbonate alkalinity, (mg/litre)} = \frac{2A \times 1000}{\text{mL of sample}}$$

(e) When $A = 0$

$$\text{Total alkalinity, (of Bicarbonate)}_{\text{(mg/litre)}} = \frac{B \times 1000}{\text{mL of sample}}$$

HARDNESS

Hardness of water is categorised into 2-classes:

(a) Temporary Hardness: It is due to the presence of bicarbonates of Ca^{2+} and Mg^{2+}. It may be removed by boiling the water.

(b) Permanent Hardness: This hardness cannot be removed by boiling the water. It is due to sulphates and chlorides of Mg^{2+} and Ca^{2+}. In general term, the Ca^{2+} hardness of water is due to the salts of calcium, magnesium, strontium, iron and manganese. The following cations and anions are responsible for the hardness of water:

Cations	Anions
Mg^{2+}	HCO_3^-
Ca^{2+}	SO_4^{2-}
Sr^{2+}	Cl^-
Fe^{2+}	NO_3^-
Mn^{2+}	SiO_3^{2-}

Reagents

1. **Erichrome Black *T* indicator:** This is prepared by mixing together 0.5 g of Erichrome black *T* and about 100 g of NaCl in 20 mL of water by warming. The solution is stable for 100 days.

2. **Ammonia Buffer:** Take 16.9 g NH_4Cl in 143 mL of liquor ammonia and dilute to 250 mL with distilled water. In presence of metallic ions, use borate buffer. Take 20 g of borax ($Na_2B_4O_7 \cdot 10H_2O$) in 400 mL of distilled water. Dissolve 5 g of NaOH and 2.5 g of sodium sulphide in 50 mL of distilled water, mix with borax solution and dilute to 500 mL with distilled water.

3. **Standard EDTA solution (0.01 N):** Ethylene diamine tetra-acetic acid disodium salt (versenate) is prepared by dissolving 2 g in distilled water to which 0.05 g magnesium chloride ($MgCl_2 \cdot 6H_2O$) is added and diluted to 1 litre.

Method

1. Take 100 mL of sample in a conical flask and add 1 mL of ammonia buffer and 2 drops of Erichrome black *T* indicator. Shake the solution well.

2. Titrate it with standard (0.01 N) EDTA solution. The colour change is from wine red to blue or bluish green.

3. At the end point no tinge of red colour should remain.

4. Note the volume used of EDTA solution from burette. This is the end point reading.

Calculation

Hardness, (mg/litre) as $CaCO_3$ can be obtained by following formula:

$$\text{Hardness (mg/litre)} = \frac{\text{Volume of EDTA used(mL)} \times \text{N} \times 1000}{\text{mL of sample taken}}$$

where, N = Normality of EDTA solution.

CHLORIDES

Chloride is invariably present in small amounts in almost all natural waters and its content goes up appreciably with increasing salinity. The estimation of chloride may be carried out by Mohr's method, when the electrical conductivity of water sample is greater than one *dS*/m at 25 ⊃C.

For public health, chlorides upto 250 mg/litre are not harmful but increase of chlorides beyond this are indication of organic pollution.

Reagents

1. **0.02 N sodium chloride:** 1.170 g of NaCl (*AR* grade dried at 80⊃C for 1 hour) is dissolved in double distilled water and made to one litre.

2. **0.02 N silver nitrate:** 3.40 g of silver nitrate is dissolved in double distilled water and made up to one litre. This is to be standardised against the standard NaCl solution and stored in amber (brown) coloured bottle, away from light.

3. **Potassium chromate indicator:** 5% aqueous solution of pure K_2CrO_4.

Method

1. Take 5 mL of sample in a porcelain dish and dilute it to about 25 mL with distilled water.

2. Add 5 to 6 drops of K_2CrO_4.

3. Titrate with standard $AgNO_3$ solution with stirring, till the first brick red tinge appears.

4. Note down the volume of $AgNO_3$ solution used from burette.

Calculations: Chloride in milliequivalent/litre

$$= \frac{\text{Normality of } AgNO_3 \times \text{volume of } AgNO_3 \times 1000}{\text{mL of sample taken}}$$

Chloride in gram/litre

$$= \frac{\text{Normality of } AgNO_3 \times \text{Volume of } AgNO_3 \times \text{Eq. weight of } Cl^- \ (35.5)}{\text{mL of sample taken}}$$

SULPHATE

Sulphates are generally found in hard water. It also indicates pollution in water.

Sulphate can be determined gravimetrically, colourimetrically, and turbedimetrically. In the oldest method, sulphates in solution are quantitatively precipitated in HCl medium and estimated gravimetrically as $BaSO_4$.

The procedure given here is based on EDTA titration described by Jackson (1973). The sulphate content in most of the irrigation water may be quite low and therefore a large volume of sample has to be first concentrated by evaporating to about 100 mL.

Reagents

1. **0.02N $MgCl_2$, $BaCl_2$ and EDTA :** AR grade $BaCl_2$ (2.44g/litre) can be directly weighed out and dissolved. $MgCl_2$ being highly hygroscopic, the required quantitiy of *AR* magnesium metal or $MgCO_3$ may be dissolved in a little excess of dilute HCl and made up to the volume. The EDTA solution must be standardized against 0.02 N $CaCl_2$.

2. **Buffer Solution :** This consists of 8.25 g of NH_4Cl plus 115 mL of NH_4OH (sp.gr.0.88) in a litre. The amount of NH_4OH may be so adjusted that 10 mL of this solution added to 50 mL of water sample can give a pH of 10.

3. **Standard $CaCl_2$ solution:** 0.02 N solution is prepared by dissolving 1.001 g of dried $CaCO_3$ (*AR*) in minimum quantity of dilute (1 + 3) HCl and made upto 1 litre with water.

4. **Erichrome Black *T* indicator:** 0.5 g of the indicator and 4.5 g of hydroxylamine hydro chloride (*AR*) dissolved in 100 mL of 95% ethyl alcohol.

Method

The method for determination of sulphates is based on the fact that a sufficient amount of standard $BaCl_2$ solution is added to the sample and back titrating the excess of barium left unprecipitated.

Take 100 mL of sample and add a few drops of methyl orange indicator and slight excess of HNO_3. Boil the mixture to remove dissolved CO_2. Add 10 mL of standard $BaCl_2$ solution in the boiling solution. Allow to cool down and make the volume upto 150 mL of clear supernatant liquid into a beaker, Add 1 mL of buffer solution and some amount of Erichrome Black T indicator. Titrate with EDTA solution until a permanent blue colour is produced indicating end point.

Calculation

Suppose 25 mL of sample is taken for precipitation of sulphates then the following formula can be used.

10 mL of $BaCl_2$ solution = 10 gm of $CaCO_3$ = 10 mL of EDTA solution

$$SO_4^{2-}\ (\text{mg/liture}) = \left[\begin{array}{l}\text{Titre value in hardness} \\ \text{estimation}\end{array} + \begin{array}{l}\text{Value equivalent to volume} \\ \text{of } BaCl_2 \text{ used}\end{array} - \begin{array}{l}\text{Titre value in sulphate} \\ \text{determination}\end{array}\right] \times 0.98 \times \frac{1000}{25}$$

Note: If the sulphate value in water is more than 100 mg/litre then volume of barium chloride solution should be increased.

FLUORIDES

Fluorides are more commonly found in ground water than in surface waters. The main sources of fluoride in water are different fluoride bearing minerals like apatite and mica. The maximum permissible limit of fluoride in drinking water is recommended to be 1.5 mg/L by WHO.

Materials and Reagents

1. **Spectrophotometer**

2. **Alizarin red Solution:** Dissolve 354 mg of zirconyl chloride octahydrate ($ZrOCl \cdot 8H_2O$) in 600 mL of distilled water. Add slowly 33.3 mL of concentrated sulphuric acid followed by 100 mL of concentrated HCl acid. Cool and further add distilled water to make the volume 1 litre.

3. **Standard Fluoride Solution:** Dissolve 221 mg of sodium fluoride in distilled water and make the volume 1 litre. This stock solution contains 10 mg F/L. Prepare a series of standard fluoride solutions by taking 0, 10, 15, 20, 25, 30, 35, 40, 45, 50 ml stock solution in volumetric flask and diluting it to 100 ml. These contain 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 mg F/L respectively.

Method

1. Take 100 mL sample in a flask and add 5 mL each of alizarin red solution and zirconyl acid solution.

2. Wait for 1 hour and then note the absorbance on spectrophotometer at 520 nm.

3. Run blank using distilled water.

Preparation of standard curve

1. Run the standard fluoride solutions of various concentrations in similar manner and record absorbance for each.

2. Plot standard curve between concentrations and absorbances obtained for standard solutions.

3. Determine the fluoride content of the sample by comparing its absorbance with standard curve and express the result as mg F/L.

SILICA

Silica in water is present as silicate. Its concentration in natural water is considerably high, ranging from 1 to 30 mg SiO_3 – Si/L or still more.

Silica is an important structural constituent for diatoms and many sponges. The assimilation of silica and subsequent sedimentation by diatoms is the major source of silica in water.

Materials and Reagents

1. **Spectrophotometer**

2. **Hydrochloric acid (50%) :** Add 50 mL concentrated hydrochloric acid to 50 mL of distilled water.

3. **Ammonium molybdate solution (10%) :** Dissolve 20 g of ammonium molybdate in distilled water and make the volume to 200 mL. Adjust the pH between 7 and 8 by adding ammonium hydroxide. Keep the solution in a polyethylene bottle.

4. **Oxalic acid Solution (10%) :** Dissolve 20 g of oxalic acid in distilled water and make the volume upto 200 mL.

5. **Standard Silica Solution :** Add 0.6714g of sodium fluorosilicate in a little distilled water and heat to dissolve. Further add distilled water to make the volume 1 litre. This stock solution contains 100 mg SiO_3–Si/litre. Dilute the stock solution with distilled water to prepare a series of standard silica solution (take 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mL stock solution in 100 mL of volumetric flask and make the volume with distilled water. This will

give working standards having 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg SiO_3 – Si / litre).

Method

1. Take 50 mL of sample in an Erlenmeyer flask and add 1 mL of hydrochloric acid and 2 mL of ammonium molybdate solution.

2. Wait for about 10 minutes and add 1.5 mL of oxalic acid solution.

3. Mix thoroughly and record the absorbance on spectrophotometer at 410 nm.

4. Carry out blank with distilled water.

5. Run standard silica solutions in similar manner and record absorbance readings. Plot a standard curve between absorbance and concentrations of standard solutions. Deduce the silica content of sample from the standard curve.

6. Express results in mg SiO_3 – Si / litre.

PHOSPHATES

Domestic and industrial effluents and agricultural run off are the major source of phosphorus in water hence its high concentration is indicative of pollution. In water although phosphorus occurs both in inorganic and organic forms but over 85% of total phosphorus is usually present in organic forms.

Principle: Orthophosphates form a heteropoly acid (phosphom acid) by reacting with ammonium molybdate and potassium antimony tartrate in acid medium. The phosphomolybdic acid reduces to molybdenum blue by ascorbic acid.

Materials and Reagents

1. Spectrophotometer.

2. Perchloric acid 70%.

3. Phenolphthalein indicator : Dissolve 1.0 g of phenolphthalein in 100 mL of ethylalcohol and 100 mL of distilled water.

4. NaOH solution (1 N) : Dissolve 4 g NaOH in distilled water and make volume to 100 mL.

5. Reagent A : Weigh 1 g of ammonium molybdate and 0.02 g of potassium antimonytartrate in 1000 mL volumetric flask. Add 16 mL of concentrated H_2SO_4 slowly by touching outlet point of measuring cylinder to the inner neck of the flask. Add distilled water slowly, shake, and make the volume upto the mark.

6. Reagent B : Weigh 0.88 g of ascorbic acid and dissolve in 1 litre of reagent *A*. It should be prepared fresh.

7. Standard Phosphate Solutions : Dissolve 2.194 g of dried anhydrous potassium hydrogen phosphates in distilled water and make upto 500 mL mark. Take 10 mL of this solution and add distilled water to make 1 litre of stock containing 1 mg P/l. Prepare standard phosphorus solution of various strengths (preferably in the range of 0.0 to 1.0 mg P/l at intervals of 0.1 mg P/l) by diluting the stock solution with distilled water.

Method

1. Take 25 mL sample in an Erlenmeyer flask and evaporate to dryness.

2. Cool and dissolve the residue in 1 mL of perchloric acid.

3. Heat the flask gently, so that the content becomes colourless. Cool and add 10 mL distilled water and 2 drops of phenolphthalein indicator.

4. Titrate against sodium hydroxide solution until pink colour appears. Make up the volume to 25 mL by adding distilled water.

5. Transfer this into 50 mL volumetric flask and add 10 mL of reagent B.

6. Make the volume to 50 mL with distilled water and let the blue colour develop. Wait for 30 minutes and record the absorbance on spectrophotometer at 660 nm.

7. Run simultaneously a distilled water blank in similar manner.

Process the standrd phosphorus solutions of different strengths in similar manner and plot a curve between absorbance and concentration of standard phosphorus solution. Deduce the phosphorus content of sample by comparing its absorbance with standard curve and express the result ot total phosphorus in mg/litre.

Calculation :

$$P \text{ mg} / l = \frac{\text{mg P in 50 mL}}{\text{volume of sample}} \times 1000$$

Here volume of sample is 25 mL.

DIFFERENT FORMS OF NITROGEN

Nitrogen is found in small amounts in water because of low solubility of molecular nitrogen in water. Only a few procaryotes can utilize this molecular nitrogen in biosynthesis. Besides this, nitrogen also occurs in small amount in water in bound forms, like ammonia, nitrites, nitrates and organic nitrogen (viz; urea, aminoacids, nucleic acids etc). The sources of such nitrogen are both autochthonous and allochthonous.

Ammonia : Ammonia is extremely soluble in water reacting with water to produce ammonium hydroxide, which further dissociates in water as ammonium and hydroxyl ions. It is one of the transient constituents in water and is a part of nitrogen cycle.

Nitrite : Nitrite is the partial form of nitrogen found in very low concentration in natural water. It has no mineral source in water, but occurs as an intermediate form during denitrification and nitrification reaction. As such, nitrite in water is formed either by oxidation of ammonia (by aerobic nitrifying bacteria, *e.g.*, Nitrosomonas) or by reduction of nitrates (by facultative anaerobic denitrifying bacteria, *e.g.*, Pseudomonas). Presence of even minute quantity of nitrite in water is indicative of organic pollution and prevailing low oxygen concentration. At high concentration it may cause blue-baby disease.

Nitrate : Beneficial effect of nitrate on crop production has been reported specially in brackish waters. The presence of K^+ and NO_3^- ions in appreciable amounts has been found to partially counteract the effect of salinity and sodium hazards of irrigation water on crop growth.

Nitrate is the most oxidised form of nitrogen and in water its most important source is biological oxidation of nitrogenous organic matter of both autochthonous and allochthonous origin. Domestic sewage and agriculture run off are the chief sources of allochthonous nitrogenous organic matter. Metabolic wastes of aquatic community and dead organisms add to the autochthonous nitrogenous organic matter. In ground water nitrates may find

way through leaching from soil. The high concentration of nitrate in water is indicative of pollution. This is an important plant nutrient. High nitrate content (> 40 mg NO_3–N/L) may cause blue baby disease.

ORGANIC NITROGEN (Total, dissolved and particulate)

Organic nitrogen is found in two forms in water, (a) particulate and (b) dissolved. Particulate Organic Nitrogen (PON) occurs as bound nitrogen in the protoplasm of aquatic organisms, while dissolved organic nitrogen (DON) occurs as products like amino acids and urea etc.

ANALYSIS OF DIFFERENT FORMS OF NITROGEN

Nitrogen (Ammonia, Nitrites, Nitrates and Organic Nitrogen)

(1) **Ammonia:** Ammonical-Nitrogen is determined colorimetrically as well as volumetrically as described below:

1. COLORIMETRIC METHOD

Materials and reagents

1. Spectrophotometer

2. **Phenol nitroprusside solution:** Dissolve 30 g of phenol in 1000 mL of distilled water. Add 2 mL of freshly prepared 1.5% w/v aqueous solution of sodium nitroprusside.

3. **Alkaline hypochlorite, solution:** Dissolve 20 g of sodium hydroxide in some distilled water and add 5.4 mL of 10% solution of hypochlorite. Make the volume to 1000 mL with distilled water.

4. **Standard ammonium chloride solutions:** Dissolve 1.91 g of anhydrous ammonium chloride in distilled water and make volume to 500 mL. This stock solution contains 1 gNH_4^+-N/L (or 1.22 g NH_3/L). Prepare a range of standard solutions by taking stock solution as 0, 1, 2, 3, 4, 5, and 6 mL and diluting it with distilled water to 100 mL. These working standard solutions contain 0, 10, 20, 30, 40, 50 and 60 mg NH_4^+-N/L.

Note : All reagents are to be prepared in ammonia-free distilled water. To remove even the traces of ammonia, distil the water after adding a small quantity of sulphuric acid to it.

Method

1. Take 40 mL of sample in a 50 mL volumetric flask.

2. Add 4 mL each of phenolnitroprusside solution and alkaline hypochlorite solution.

3. Make up the volume of contents to 50 mL by adding ammonia-free distilled water.

4. Keep it in a dark place at 25 ⊃ C for about 1 hour.

5. Record the absorbance on spectrophotometer at 625 nm. Use distilled water as blank.

6. Process the standard ammonium chloride solutions of different concentrations in similar way and record the absorbance for each.

7. Plot the concentration of ammonium ions in sample in mg NH_4^+ N/L against absorbance and prepare standard curve. Calculate concentration of unknown with the help of curve.

Materials and Reagents

1. Micro-Kjeldhal distillation assembly.

2. **Hydrochloric acid (0.01 N):** Dilute 8.34 mL of 12 N concentrated hydrochloric acid with distilled water to prepare 100 mL of 1.0 N hydrochloric acid. Dilute 10 mL of this 1.0 N HCl with distilled water to prepare 1 litre of 0.01 N HCl.

3. **Boric acid cum indicator solution:** Dissolve 4 g of boric acid in 100 mL of warm distilled water. Prepare 0.5% bromocresol green solution and 0.1% methyl red solution in ethyl alcohol. Mix bromocresol green and methyl red solutions in the ratio of 2 : 1 to make a mixed indicator. Add 5 mL of this mixed indicator to 100 mL of boric acid solution. If the colour of solution becomes blue, add 0.01 N hydrochloric acid until it turns faint pink to brown.

4. **Borax buffer solution:** Add 4 g of borax crystals to 100 mL of distilled water and heat to dissolve.

Method

1. Take 50 mL of sample in micro-Kjeldhal distillation flask and add 1 mL of borax buffer solution.

2. Put 5 mL of boric acid cum indicator solution in a conical flask. Place it below the condenser so that the tip of outlet of condenser is dipped in contents of conical flask.

3. Heat the Kjeldhal flask containing water sample.

4. Continue distillation until about 40 mL of distillate is collected in thc conical flask.

5. Remove the conical flask having distillate, which turns blue due to dissolution of ammonia.

6. Titrate the distillate in conical flask against 0.01 N hydrochloric acid. Turning of blue colour to faint pink or brown indicates the end point.

7. Run a blank with distilled water in a similar way.

Calculation:

$$NH_4 - N\ (mg/L) = \frac{(T - B) \times N \times 1000 \times 14}{\text{Aliquot taken (mL)}}$$

where, T = volume of titrant used against sample (mL);

B = volume of titrant used against blank (mL);

N = normality of titrant (0.01). The equivalent weight of NH_4-N = 14.

Note: Ammonia in the water is released as an end product of decomposition of organic matter and also as an excretory product of some aquatic animals. It dissolves in water to form ammonium hydroxide which further dissociates into ammonium (NH_4^+) and hydroxyl (OH^-) ions. Domestic wastes being often rich in nitrogenous organic matter and many industrial effluents add to the ammonia load in water leading to toxic levels at certain times. Aquatic autotrophs are capable of utilizing ammonium ions at a fast rate.

2. NITRITE

Nitrites can be determined colorimetrically by EDTA method and sulphanilamide method. The EDTA method is described in the following paras:

EDTA Method

Materials and Reagents

1. Spectrophotometer

2. **EDTA solution:** Dissolve 0.5 g of disodium salt of EDTA (ethylene diamine tetra-acetic acid) in distilled water to prepare 100 mL of solution.

3. **Sulphanilic acid solution:** Dissolve 600 mg of sulphanilic acid in about 70 mL of warm distilled water. Cool, add 20 mL of concentrated hydrochloric acid, and make the volume of content to 100 mL by further adding distilled water.

4. **α-Naphthylamine hydrochloride solution:** Dissolve 0.6 g of α-naphthylamine hydrochloride in a little distilled water. Add 1 mL of concentrated hydrochloric acid and make the volume to 100 mL by further adding distilled water. If on storage a precipitate appears, filter the reagent before use.

5. **Sodium acetate solution:** Dissolve 27.2 g of sodium acetate in distilled water to make 100 mL of solution.

6. **Standard nitrite solutions:** Dissolve 1.232 g of sodium nitrite in distilled water and make volume to 1 litre. Take 4 mL of this solution and again make volume to 1 litre with distilled water. This stock solution has 1 mg NO_2-N/litre (or 2.290 mg NO_2 ions/litre).

7. **Preparation of standard curve:** Prepare standard nitrite solutions of various concentrations by taking 0, 10, 20, 30. 40, 50, 60, 70 and 80 mL. of stock solution and dilute it to 100 mL with distilled water. These working standard solutions contain 0.0. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg NO_2-N/L.

Method

1. Take 50 mL filtered sample in an Erlenmeyer flask and add 1 mL of each EDTA solution, sulphanilic acid and α-naphthylamine hydrochloride solution one after the other. Appearance of wine red colour indicates the presence of nitrites.

2. Record the absorbance of this solution on spectrophotometer at 520 nm.

3. Carry out blank with distilled water

4. Run standard nitrite solutions in similar way and record the absorbance for different concentrations. Plot a standard curve between absorbance and concentrations of standard solutions and deduce the nitrite-nitrogen content of sample by comparing its absorbance with the standard curve. Express the result as mg NO_2-N/L.

3. NITRATE

The nitrate in water can be determined by phenol disulphonic acid method.

Phenol disulphonic acid method

Principle

Nitrate in contact with sulphuric acid produces nitric acid which, in dry condition (in presence of excess conc. H_2SO_4), brings about nitration of phenol disulphonic acid. This nitrophenolic product gives intense yellow colour in alkaline medium which is measured through colorimeter (the reaction must proceed in cold otherwise nitric acid may be lost by volatilization).

Materials and Reagents

1. Spectrophotometer
2. Hot water bath
3. **Phenol disulphonic acid:** Dissolve 25 g of white phenol in 150 mL of concentrated sulphuric acid then again add 85 mL of concentrated sulphuric acid. Heat it for about 2 hours on a water bath, cool, and keep the solution in a dark bottle.
4. **Liquor ammonia (LR grade):** It is diluted with equal volume of water.
5. **Standard nitrate solutions:** Dissolve 0.722 g of anhydrous potassium nitrate in distilled water to prepare 1 litre of stock solution. This stock solution contains 100 mg NO_3-N/L (or 443 mg NO_3 ions/litre).

Method

1. Take 25 mL of sample in a porcelain dish (50 mL capacity) and evaporate it to dryness on a hot water bath (if porcelain dish is not available take 50 mL beaker or silica dish).
2. Add 3 mL of phenol disulphonic acid to the residue and dissolve the latter by rotating the dish.
3. After ten minutes, 15 mL of distilled water is added and stirred with a glass rod. On cooling, the contents are washed down into 100 mL volumetric flask.
4. Add ammonia (1 : 1) slowly with mixing till the solution is alkaline as indicated by the development of yellow colour due to the presence of nitrate. Then add another 2 mL of ammonia and the volume made up (100 mL) with distilled water.
5. Intensity of yellow colour is read in the colorimeter at 420 nm (blue filter).
6. Add 5 mL of distilled water and 1.5 mL of potassium hydroxide solution.

Observations

Preparation of Standard Curve for Nitrate

A stock solution containing 100 ppm nitrate-nitrogen (NO_3^-) is prepared by dissolving 0.7215 g of AR grade potassium nitrate (oven-dried and cooled) in distilled water and making the volume to one litre. This is diluted ten times to give a 10 ppm NO_3^- solution. Aliquots (2, 5, 10, 15, 20 and 25 mL) are evaporated on boiling water bath to dryness in small porcelain dishes (or beakers). When cool, 3 mL of phenol disulphonic acid is added and yellow colour is developed and read as described above. A blank (without nitrate) must be run and correction made by adjusting the colorimeter to zero with blank. A calibration curve is drawn between concentration of NO_3^- and colorimeter reading.

Calculations : mg of NO_3^-/litre $= \dfrac{\mu g \text{ of } NO_3^- \text{ from standard curve}}{\text{mL of sample}}$

4. TOTAL ORGANIC NITROGEN (TON)

Materials

1. Micro-Kjeldahl distillation assembly.

2. **Digestion mixture :** Dissolve 16.25 g of potassium sulphate in 200 mL of distilled water. Add 0.4 g of mercuric oxide and slowly 25 mL of concentrated sulphuric acid. Further, add distilled water to make the volume 250 mL.

3. **Hypo solution :** Dissolve 50 g of sodium hydroxide in 200 mL of distilled water and add 10 g of sodium thiosulphate. Make up the volume to 250 mL by adding distilled water.

4. **Boric acid solution :** Dissolve 1 g of boric acid in distilled water to make 100 mL of solution.

5. **Mixed indicator :** Prepare 0.1% methyl red solution and 0.5% bromocresol green solution in 95% ethyl alcohol. Mix the methyl red and bromocresol green solutions in 1 : 2 ratio.

6. **Hydrochloric acid (0.01 N) :** Dilute 8.34 mL of concentrated hydrochloric acid with distilled water to prepare 100 mL of 1.0 N hydrochloric acid. Dilute 100 mL of this 1.0 N HCl with distilled water to prepare 1 litre of 0.1 N HCl. Take 100 mL of 0.1 N HCl and dilute to 1 litre with distilled water to get 0.01 N HCl.

Method

1. Take 200 mL of sample in an evaporating dish and evaporate to dryness.
2. Add 4 mL of digestion mixture to the residue and dissolve it in about 20 mL of distilled water.
3. Heat the solution to fuming for over 15 minutes and cool.
4. Transfer the digest to micro-Kjeldahl distillation assembly and add about 3–5 mL of hypo solution.
5. Take 5 mL of boric acid solution containing 2–3 drops of mixed indicator in a conical flask and place the flask below the condenser so that the tip of outlet of the condenser is dipped in contents of conical flask.
6. Heat the Kjeldahl flask. Continue distillation for about 10 minutes. Remove the conical flask having distillate.
7. Titrate the distillate against hydrochloric acid. End point is determined by change of blue colour to pink colour.
8. Also run a blank using distilled water in similar way.

Calculation:

$$\text{T.O.N.}\,(\text{mg / L}) = \frac{(T - B) \times N \times 1000 \times 14}{\text{Volume of aliquot}}$$

where, T = Volume of titrant (HCl) used against sample (mL),
B = Volume of titrant (HCl) used against blank (mL),
N = Normality of titrant (0.01)

The atomic weight of N is 14.

5. DISSOLVED ORGANIC NITROGEN (DON)

Filter the sample through a millipore filter paper using a filtration unit and apply the same process of estimation which was employed in total organic nitrogen (*TON*). Express the result of dissolved organic nitrogen in mg/litre.

6. PARTICULATE ORGANIC NITROGEN (PON)

Determine the dissolved organic nitrogen and total organic nitrogen as described above, calculate the particulate organic nitrogen by the formula given below:

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

HEAVY METAL POLLUTION

Water pollution problems exist everywhere in the country and are increasing day-by-day around industrial and urban centres. Waste water effluents, industrial effluents and other sources carry different types of heavy metals, which are liberally let out into the rivers causing contamination in them.

Metals and their toxicity: The power of toxicity varies from metal to metal. The word toxic is derived from Greek words toxon (bow) and toxicon or pharmikon (arrow poison). The term toxicology can be defined as, 'a branch of science which deals with the study of adverse and harmful effects of chemical agents on any biological system'.

Types of Toxicology: Toxicology is divided into five categories:

(a) Clinical Toxicology

(b) Industrial Toxicology

(c) Forensic Toxicology

(d) Environment Toxicology

(e) Economic Toxicology

(a) Clinical Toxicology: It deals with the study of diagnosis symptoms and treatment of disease which are produced by the adverse effects of chemicals and heavy metals.

(b) Industrial Toxicology: This branch deals with the study of those materials which are responsible for health hazards and industrial hygiene.

(c) Forensic Toxicology: It deals with medical and legal aspects of adverse effects of chemicals on human beings.

(d) Environmental Toxicology: This branch deals with the study of incidental exposures of man and other living beings to harmful contaminants of environment.

(e) Economic Toxicology: It deals with the study of herbicides, insecticides, pesticide and their effect on insects, pests, domestic animals and human beings.

Toxicity of Metals: Toxicity of a metal depends on the inherent capacity of a metal to affect adversely any biological activity. Toxic metal changes the biological structures and systems of the living organisms and also causes deformity in the body or finally even death. At higher concentration, almost all metals are toxic, whereas at very low concentration some are lethal. Within a certain limit, heavy metals are essential for plants, aquatic animals as well as human beings for their survival and biological functions.

We will study here the affects of some common metals found in industrial effluents and found in nature in the following paras.

1. Cadmium

Occurrence: Cadmium does not exist free in nature and there is no specific ore from which it can be obtained. Cadmium is obtained from refining of zinc, and copper as by-product. In plants and sea animals, cadmium is found in trace amounts. In wheat and rice, cadmium is present in appreciable amount. Cigarette tobacco contains 70% cadmium content.

Uses

1. Cadmium is used in industries as protective coating for iron, steel and copper.

2. Cu-Cd alloys are used for manufacturing telephone wires.

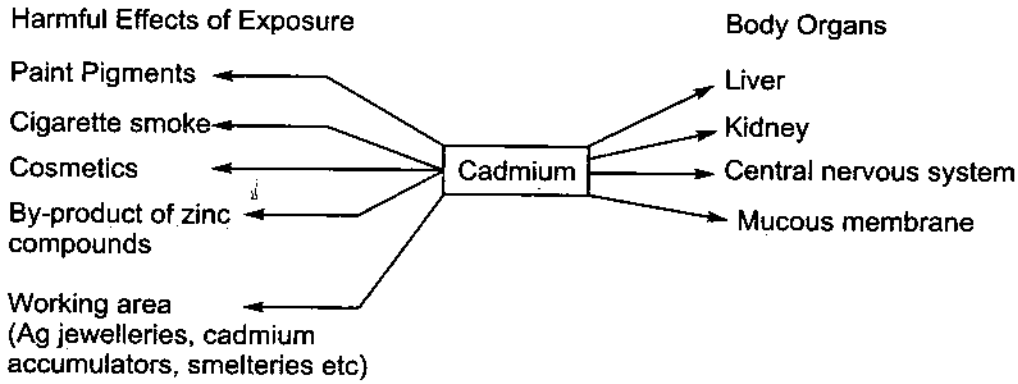
3. In electronic equipments Ni—Cd batteries are used.

Toxicity and diseases: Cadmium is toxic to all human beings and animals. Cadmium dust, fumes and mists pollute the atmosphere. Cadmium toxicity of even 50 mg quantity causes various diseases in man such as:

(a) Cadmium toxicity is responsible for vomitting, diarrhoea, abdominal pains and loss of consciousness.

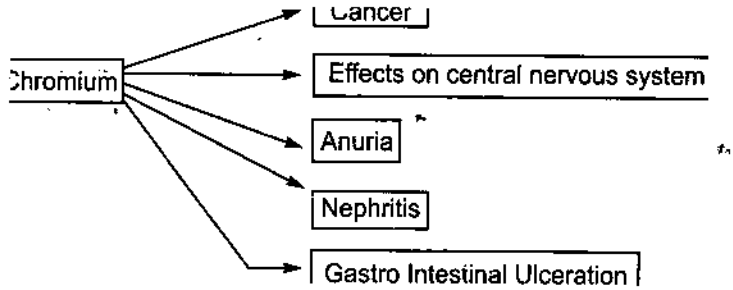
(b) It also causes redardation of growth, deformity in bones, hypertension, impaired kidney functioning, impaired reproductive function, formation of tumor and teratogenic effects.

(c) Inhaling of cadmium dust or fumes causes choking of nose, coughing, bronchitis and damage to respiratory tract. Effects of cadmium poisoning are given below:



2. Chromium

Occurrence : In nature, chromium occurs as chrome iron ore ($FeO \cdot Cr_2O_3$). It is also found in soil and plants. It is present in earth's crust at about 200 ppm and in sea water at 1.0–2.5 ppb. In human body, chromium accumulates mainly in lungs with age. The quantity of chromium in a human is about 6 mg. chromium attached with the protein β-globulin and distributed in different parts of body like spleen, testes, liver, brain, heart and lungs.



Diseases of chromium poisoning

Uses : Chromium is used in industries such as

(a) Steel manufacturing

(b) Photographic work

(c) Production of Jet engines

(d) Preparation of paints, electric cells, matches

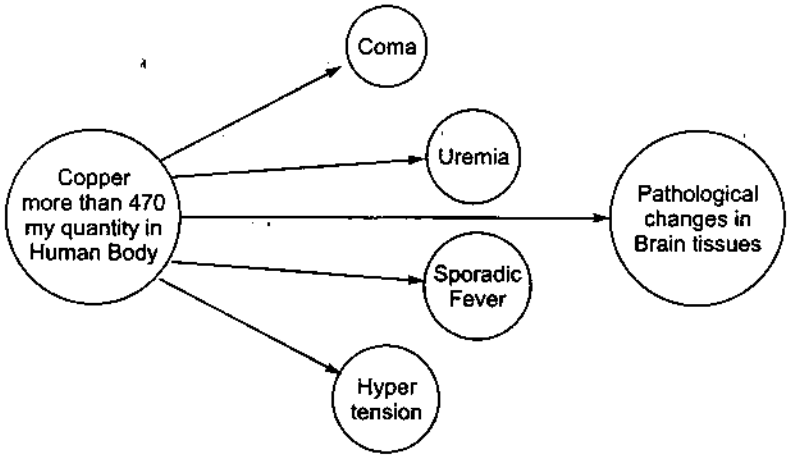
(e) Manufacturing of rubber goods and tools etc.

Toxicity and Diseases : Trivalent chromium is least toxic of trace elements whereas hexavalent chromium more than 70 mg in quantity is highly toxic in nature.

3. Copper

Occurrence: In nature, copper occurs in sulphide ores. It is found in the form of native metal. In the human body, copper is present in liver and brain. Copper is found in some plants and animals. In black pepper it is present as 53 ppm whereas in oysters its quantity is 137 ppm.

Toxicity and Diseases: Copper is an industrial health hazard. More than 470 mg copper in human body is toxic and causes many disorders in the body such as :



Effects of copper in human body

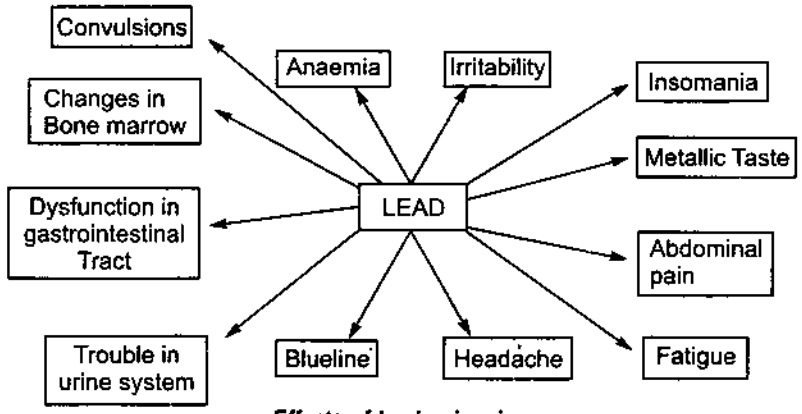
4. Lead

4. Occurrence: In the nature, lead is found in bound form of ores. These ores are galena, cerussite ($PbCl_2$) and its sulphide. Lead is found in all living organisms. In a human body about 96% lead is present in the bones. The concentration of lead increases with age, as the amount of lead in an overage human body is 120 mg but it may increase upto 400 mg. Lead is not an essential metal for mammalians. Lead is also present at 15 ppm in earth's crust. In marine water the amount of lead is 5 ppb.

Lead is usually deposited in bones and some soft tissues. It is also retained by animals in liver, kidney, muscles etc.

Under some specific conditions lead becomes stimulatory and it enhances (a) protein synthesis (b) DNA synthesis (c) cell replication

Toxicity and Diseases : About 800 mg lead creates toxicity in human beings resulting in lead poisoning. Due to lead poisoning a number of body disorders are caused, some or which summarised below:



Effects of lead poisoning

When the amount of lead in human body is more than 500 mg, it shows the toxicity symptoms which are mild anaemia, brain damage, vomiting, loss of appetite, convulsions, unco-ordinated body movements and stupor, eventually producing coma and even, causes death.

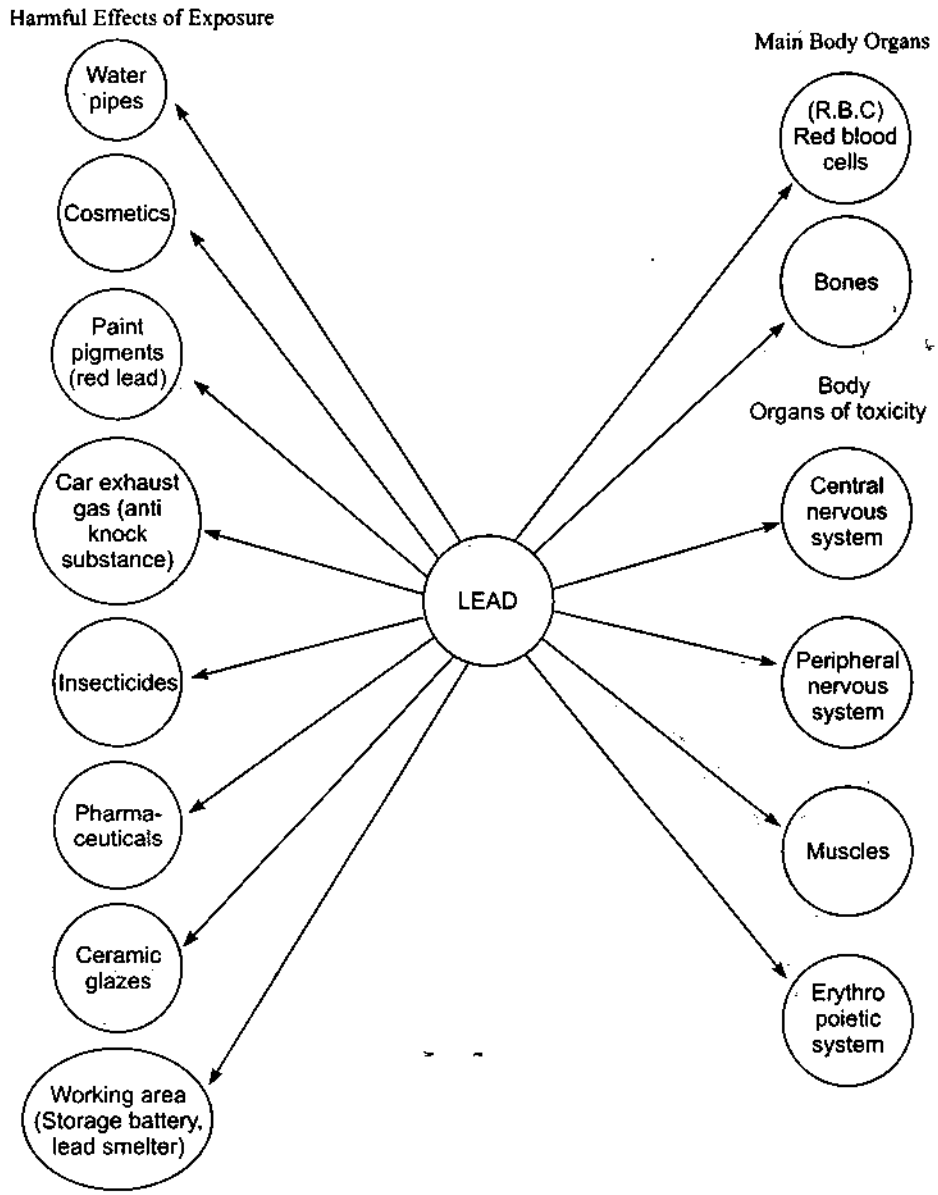
Generally, lead toxicity is due to concentration of diffusion of Pb in soft tissues. Another possible common mechanism for Pb toxicity is due to formation of metallothionein. Effects of lead poisoning are summarised here and hazards of harmful effect of exposure of lead are given on page 130.

5. Zinc

Occurrence: Zinc is not found in free form, it is present in ores. These ores are (a) blende, (b) sulphide ore, (c) silicate ore, (d) zinc spar and (e) zincite ZnO etc. Zinc is present in different other sources such as:

Sea water – Zinc present as 9.21 ppb.

The human body contains 300 mg zinc which is distributed in :



Harmful Effects of Lead Exposure

(i) muscles – 65% zinc

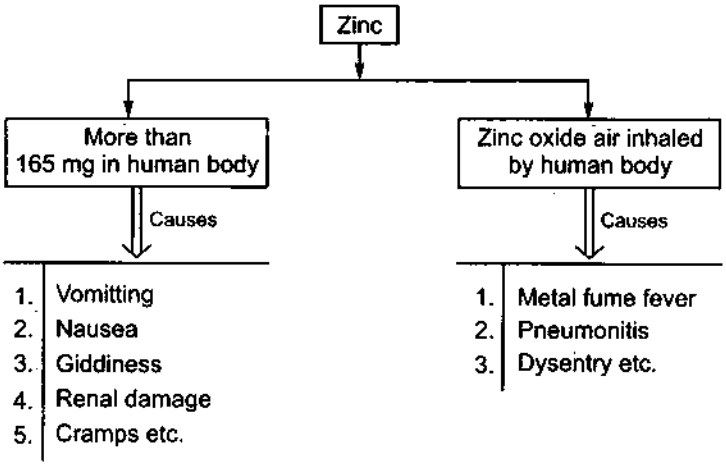
(ii) bones – 20% zinc

(iii) plasma – 6% zinc

(iv) erythrocytes – 2.8% zinc

(v) liver – 53% zinc

Toxicity and Diseases: Zinc salts are relatively non-toxic but heavy doses i.e., more than 165 mg zinc causes some disorders in human body which are summarised below :



6. Manganese

Occurence: Manganese occurs in nature as oxide of

(a) pyrolusite(b) magnatite

(c) braunite(d) tephroite (silicate)

(e) manganese spat (carbonate)

Manganese is also found in

(i) sea water – an amount of 1 ppb

(ii) in Human body – about 12 mg

(iii) in bones – 45% manganese

(iv) in soft tissues and brain

Manganese (Mn) is also found in plants. It is an essential metal for mammals.

Uses: Manganese is used in industries in the manufacture of

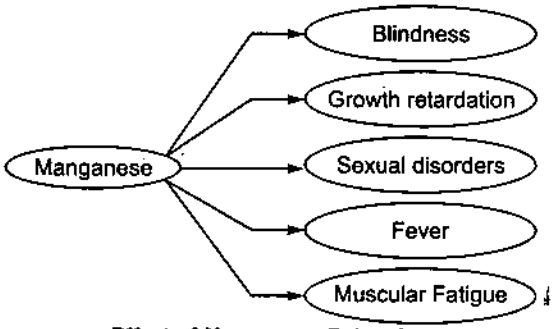
(a) alloys

(b) dry cell batteries

(c) fire works

(d) glass and ceramic industries

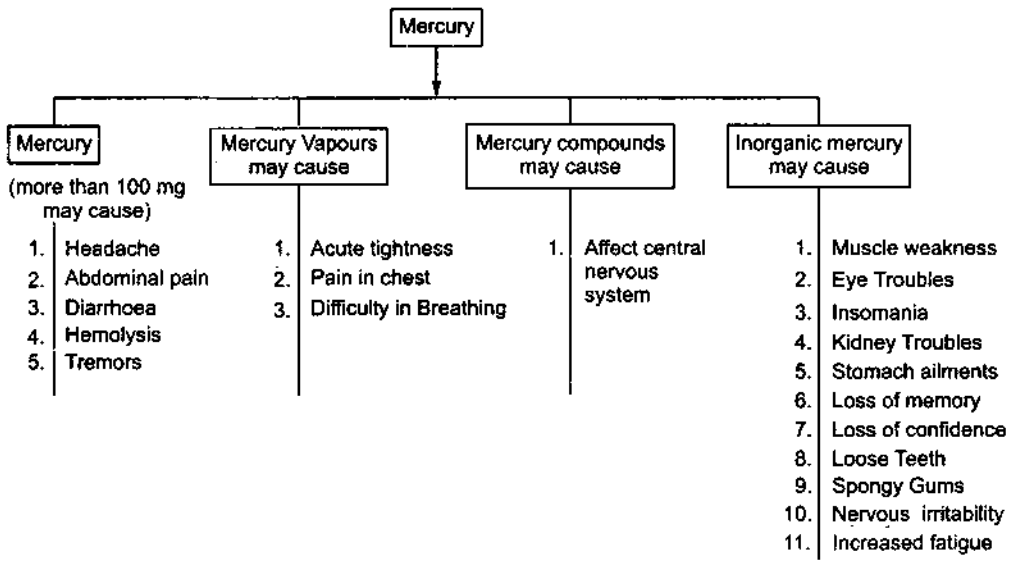
Toxicity and Diseases: Manganese is least toxic out of essential metals. Permanganate is found most toxic among manganese forms. Divalent Mn^{2+} is three times more toxic than trivalent Mn^{3+}. In human body, ingested Mn^{2+} is converted into Mn^{3+}. Manganese absorption is increased by Fe deficiency. Manganese salts are deposited in lungs which are absorbed there slowly. Manganese is toxic when its concentration is more than 100 ppm in human body. It causes following disorders:



Effect of Manganese Poisoning

7. Mercury

Occurrence: Mercury occurs as native metal mixed with its ores. The human body contains about 13 mg mercury, 70% of which is present in muscle tissues. Soft tissues absorb very high amount of mercury. In human body mercury is found in kidney, liver, intestinal and colon walls, brain, heart, lungs, respiratory mucosa, muscles and skin. Body tissues absorb mercury more in the form of CH_3Hg^+ incomparison of Hg^{2+} salts.



Toxicity and Diseases : Mercury and its salts are severe health hazards. Mercury is very toxic in nature. Its amount of more than 100 mg causes body disorders. Mercury compounds as well as its vapours are harmful for human body. Inorganic form of mercury is also injurious to health. The effects of mercury poisoning are as follows :

Mercury is converted into methyl mercury salt and dimethyl mercury by microorganisms, which escape into atmosphere. With the help of bacteria and ultravoilet light, above reaction occurs in sediments of rivers and sea beds.

Dimethyl mercury is also converted into free toxic elemental mercury. Methyl mercury (CH_3Hg^+) has covalent bonding therefore it is more stable. The methyl mercury is very toxic and can enter into cellular membrane.

$$(CH_3)_2Hg \xrightarrow[\text{Bacteria}]{\text{Ultravoilet light}} Hg + C_2H_6$$

$$(CH_3)_2Hg \longrightarrow CH_3Hg^+ \longrightarrow \text{Bacteria } CH_3Hg \text{ Thiols}$$

$$\downarrow$$

$$Hg^+ \text{ taken by fish}$$

Blood serum proteins from —S—Hg—CH_3 complex are responsible for killing of fish in rivers and oceans. Tissues and erythrocytes oxidise mercury and Hg to Hg^+ ions.

From industries, methyl mercury settles as sediment at the bottom of water bodies, from where fish trap mercury salts. If such types of mercury contaminated fish are used in food they cause neurotoxic minamata disease.

Body tissue retention is greater for CH_3Hg^+ than for Hg^{2+} salts. The toxic action is due to crowding of Hg^{2+} ions around the immediately available thiol groups of proteins and delay in distribution of these ions among rest of thiol group throughout the body.

8. Arsenic

Occurrence : Arsenic occurs in nature as a brittle metal. It is present in following forms :

(a) in marine water–5 ppb

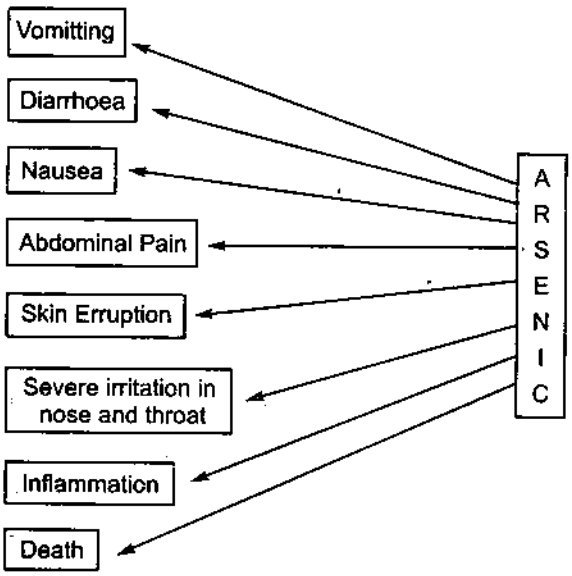
(b) in earth's crust–2 ppm

(c) in human body–about 18 mg found in tissue.

(d) in blood–about 25 mg

Arsenic is found in animal body as well as in human body particularly in hair, spleen and aorta. It is also present in erythrocytes, here arsenic binds globin part of haemoglobin. Compounds of arsenic are absorbed easily by skin of human being.

Toxicity and diseases : Arsenic trioxide As_2O_3 is more toxic than As_2O_5. Its large quantity in human body (more than 25 mg) causes many diseases as given below:



Effect of arsenic on body

Limits of some heavy metals in drinking water are prescribed by the Government of India; which are given below :

Concentration of heavy metals (µg/l) in drinking water

Metals	Permissive limit	Excessive limit
Chromium (Cr) VI	–	50
Copper (Cu)	1000	3000
Lead (Pb)	–	100
Manganese (Mn)	100	500
Zinc (Zn)	5000	15000
Arsenic (As)	–	200
Iron (Fe)	300	1000
Selenium (Se)	–	50

Trace Metals in the Raw Sewage and Treated Effluent

TraceMetals	Raw Sewage		Treated effluent	
	Range	Average	Range	Average
Boron	µg/*l* 185–315	295	100–167	135
Cadmium	µg/*l* 10–20	12	10	10
Cobalt	µg/*l* 50–200	87	50	50
Chromium (VI)	µg/*l* 12–456	29	20	20
Chromium (Total)	µg/*l* 50–200	87	25	25
Copper	µg/*l* 50–200	109	25–160	68
Lead	µg/*l* 50–200	112	2750–14750	9042
Iron	µg/*l* 840–2400	1275	405–1375	656
Manganese	µg/*l* 235–610	344	130–250	205
Zinc	µg/*l* 405–905	605	50–165	89
Nickel	µg/*l* 50–200	87	50	50
Physical Properties				
Colour	light brown	–	nil	–
Odour	nil	–	nil	–
pH	7.09–8.24	7.62	7.8–8.0	8.08
Electrical conductivity	2.86–53 milli mho per cm.	3.81	1.33–4.24	2.91

The above analytical data of trace metals in raw sewage and treated effluent indicates that heavy metals, toxic materials and other minerals can be suitably controlled within a sewage system.

ANALYSIS OF HEAVY METALS IN AQUEOUS SYSTEM

Surface water sample was taken for heavy metal analysis. This water sample was preserved in ice kits in the analytical laboratory. To remove any impurities and suspended particles, the samples were filtered through 0.45 µ sized filter membrane. These filtered samples were stored in acid–washed polyethylene bottles. Heavy metals like Cu, Mn, Fe, Zn, Cr and Ni etc were analysed in water samples (aqueous system) by Atomic Absorption Spectrophotometer using model-varian A.A.975. Some analytical data obtained from atomic absorption and colorimetric methods for heavy metals are given below :

Analytical Data of Heavy Metals by instrumental Techniques

Heavy Metals	Unit	By Atomic Absorption	By Colorimetry
Iron	µg/*l*	840	808
Chromium (total)	µg/*l*	72	66.7
Copper	µg/*l*	160	185
Zinc	µg/*l*	565	536
Manganese	µg/*l*	235	277

Determination of Heavy Metals (Zn, Cu, Fe, Mn) By Instrumental Technique using Atomic Absorption Spectrophotometer: This method was given by Lindssay and Norvell in 1978. For the extraction of zinc, copper, manganese and iron from the aqueous system, DTPA (Diethylene triamine penta acetic acid) is used in this method. DTPA is a chelating agent which combines with free metal ions in the aqueous system to form soluble complexes. Stability constant for the simultaneous complexing of Zn, Cu, Mn,

and Fe shows DTPA as the most suitable extractant. The DTPA has a capacity to complex each of the metal cations as 10 times of its atomic weight. The content of these cations is determined on an atomic absorption spectrophotometer (A.A.S.).

Apparatus

1. Analytical balance 2. Pipette – 20 mL capacity.
3. Polyethylene narrow mouth bottles – 100 mL capacity.
4. Whatman No. 1 or Whatman No. 42 filter papers.
5. Glass Funnels6. Polyethylene vials – 50 mL capacity.
7. Hollow Cathode Lamps of Zn, Cu, Fe and Mn.
8. Atomic Absorption spectrophotometer (AAS).
9. Volumetric flasks – 1 litre capacity, and 100 mL capacity.

Reagents

1. DTPA Solution: 0.005 M

Take 1.967 of DTPA in 1 litre volumetric flask and make the volume with deionized or glass distilled water up to the mark. Shake it well to mix thoroughly with stopper.

2. Stock Standard Solutions : The standard solutions of different metal cations should be prepared by using their foil or wire of *AR* grade. Dissolve 0.1g of the metal foil in dilute HCl (in ratio 1 : 1) and make the volume to one litre with deionized water to obtain 100 μg/ml} (*i.e.*, mg/L or ppm) solution of every metal cation.

Alternatively, analytical grade salts can be used to prepare stock standard solutions of different metals. The amount of the salt to be taken for its standard solution, its chemical formula, and concentration of respective stock solution is given below:

Metal	Amount of Salt to be taken in 1 litre of solution (gm)	Concentration of stock solution (μg/ml})	Salt to be used for stock solution
Cu	0.3928	100	$CuSO_4 \cdot 5H_2O$ (Copper sulphate)
Fe	0.4964	100	$FeSO_4 \cdot 7H_2O$ or (Ferrous sulphate)
	0.7028		$[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ Ferrous ammonium sulphate
Mn	0.3076	100	$MnSO_4 \cdot H_2O$ (manganese sulphate)
Zn	0.4398	100	$ZeSO_4 \cdot 7H_2O$ (Zinc sulphate)

The amount of above given salts should be dissolved in a small amount of deionized or glass distilled water. After mixing thoroughly add about 5 mL of (1 : 5) sulphuric acid. This content is then diluted to one litre in a volumetric flask with deionized or glass distilled water.

WORKING STANDARD SOLUTIONS

1. Zinc: Transfer 10 mL of stock standard solution to 100 mL volumetric flask and dilute it with DTPA solution, upto the mark. The strength of this

stock solution is now 10 μg zinc/mL (10 ppm). Take 0, 1, 2, 4, 6 and 8 mL of stock solution (*i.e.*, 10 μg zinc/mL) to a series of 100 mL volumetric flasks and dilute each upto the mark with the help of DTPA solution. This will give standard solutions having zinc concentration 0, 0.1, 0.2, 0.4; 0.6 and 0.8 μg/mL (ppm).

2. Iron: Transfer 0, 1, 2, 4, 6, and 8 mL of stock solution (100 μg Fe/mL or 100 ppm Fe) to a series of 100 mL volumetric flasks. Dilute each of the flasks upto mark with DTPA solution. This will give standard solutions having iron concentration of 0, 1, 2, 4, 6 and 8 μg/mL (ppm).

3. Copper: Transfer 0, 1, 2, 4, 6, and 8 mL of stock solution containing 100 μg Cu/mL (100 ppm Cu) to a series of 100 mL volumetric flasks and dilute each of the above solutions upto the mark with the help of DTPA solution. It will give standard solutions containing copper concentrations 0, 1, 2, 3, 4, 6, and 8 μg/mL (ppm).

4. Manganese: Transfer 0, 1, 2, 3, 4, 6, and 8 mL of the stock solution (100 μg Mn/mL or 100 ppm Mn) to a series of 100 mL volumetric flasks and dilute each to the mark with DTPA solution. These standard solutions have manganese concentrations of 0, 1, 2, 3, 4, 6 and 8 μg/mL (ppm).

Method

By taking above prepared solutions, the metals zinc, copper, iron and manganese can be determined in the aqueous samples using atomic absorption spectrophotometer (AAS). The operational technique of AAS is described below:

1. First of all set 'zero' of the instrument using blank solution.

2. Feed standards belonging to the metal to be determined to the atomic absorption spectrophotometer to standardize the instrument to read absorbance or concentration in the sample having the given metal within the standardized range.

3. Then feed the DTPA–extract and record the absorption or concentration of the metal.

4. Repeat the above steps for every metal.

5. In case the instrument shows a sign of 'over' for some metal in a particular sample indicating thereby that sample has a concentration out of the range for which the instrument has been standardized, then make further dilution of the sample by 2–5 times and feed the sample again and record the absorbance or concentration.

Calculations: Most of the modern *AASs* are calibrated to display the concentration of a metal in ppm directly in the sample. In such cases, the concentration of the given metal in the sample is calculated by multiplying the displayed reading by the dilution factor which is 2 in this case.

If the *AAS* (Atomic absorption spectrophotometer) displays the reading in absorbance, then a standard curve has to be prepared for the known standards on a graph paper, and the absorbance readings should be converted into concentration (μg/mL) from the standard curve. The amount of the 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 the *AAS* = *A*

Concentration of the metal as read from the standard curve against

A = C µg/mL*

Content of the metal cation in the sample = C × 2 mg/Kg or ppm

µg/mL = mg/kg = ppm

Precautions

1. Deionized or glass distilled water should be free from any metal cations.

2. Apparatus (glass/polyethylene) to be used for the analysis must be thoroughly washed with acidified water and then with deionized water.

3. Before feeding the solutions, it should be ensured that they are not turbid otherwise, they may block the capillary of the atomic absorption spectrophotometer.

Standard Curve

The standard curve showing relationship between metal concentration and absorbance is given below in adjacent figure 17.

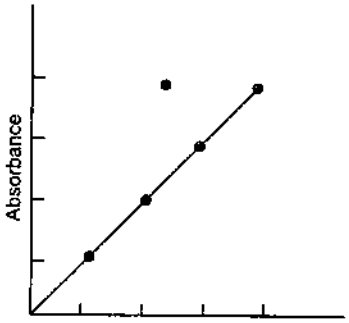


Fig. 17

Estimation of Heavy Metals Cd, Cr, Ni and Pb

Water has become polluted in many parts of the world with heavy metals such as Cd, Cr, Ni and Pb etc. The reasons of heavy metal pollution are:

(a) Disposal of sewage sludge

(b) Mining and smelting activities

(c) Disposal of other types of wastes etc.

Cadmium: Cadmium metal is a considerable health hazard to human beings as well as animals. The presence of Cd even below 1 ppm is very harmful to life.

Chromium: At higher concentration, chromium is a toxic element for mammals and other animals. Range of the pollutant varies from 10 to 100 ppm.

Nickel: 10 to 100 ppm range of nickel works as pollutant. It is a possible carcinogenic abscessing metal pollution.

Lead: Lead is a harmful metal to human and animals as environmental pollutant.

TOTAL HEAVY METALS ANALYSIS

In total analysis, a precise and quick method is applied, which includes cadmium, chromium, nickel and lead. It is done in a Teflon–lined bomb and digested for 1 hour at 100°C with aquaregia and hydrogen fluoride (HF). The details of this method are given below.

Reagents

1. Nitric acid–concentrated and 1:1 dilution in deionized water (> 10 mega ohm–cm resistivity).

2. 30% Hydrogen peroxide.

3. Hydrochloric acid–concentrated.

Method

1. Add 10 mL of 1 : 1 HNO_3 to 2 mL of water in a 150 mL beaker.

2. Keep the sample on an electric hot plate, cover with a watch glass, and reflux (heat) for 15 minutes at 95°C.

3. Cool it and add 5 mL of conc. HNO_3. Reflux for an additional 30 minutes at 95°C.

4. Repeat the last step and evaporate to reduce the solution to about 5 mL without boiling. During this time, partially cover the beaker with a watch glass.

5. Cool the sample and add 30% hydrogen peroxide (H_2O_2) in about 3 mL quantity and also add 2 mL deionized water.

6. Cover the beaker and heat the sample gently to start the peroxide reaction. If effervescence becomes excessively vigorous, remove the sample from the hot plate.

7. Continue to add 30% H_2O_2 in 1 mL increments, followed by gentle heating until the effervescence subsides.

8. Add 5 mL of conc. HCl and 10 mL of deionized water and reflux the sample for an additional 15 minutes without boiling.

9. Cool and filter the sample through a Whatman No. 42 filter paper. Dilute it to 50 mL with deionized water.

10. Analyse for Cd, Cr, Ni and Pb by atomic absorption or inductivity coupled plasma (ICP) spectroscopy.

The data given below show best detection limit of Cd, Cr, Ni and Pb Atomic Absorption (AA) and ICP-emission spectroscopy:

Method of Analysis

Metals	Flame AA	Electrothermal AA $\mu g/L^{-1}$	ICP
Cd	0.5	0.0002	0.07
Cr	2.0	0.004	0.08
Ni	2.0	0.05	0.2
Pb	10.0	0.007	1.0

DISSOLVED OXYGEN

Dissolved Oxygen (DO) in water is an index of physical and biological processes going on, non-polluted surface waters are generally saturated with dissolved oxygen. There are two main sources of dissolved oxygen in water.

1. Diffusion from air/absorption from air
2. Photosynthetic acitivity within water by vegetation etc.

Diffusion of air from air to water is a physical phenomenon and is influenced by factors which affect the oxygen solubility, like temperature, water movement and salinity etc. Photosynthetic activity within water is a biological phenomenon carried out by autotrophs (mainly phytoplanktons in water) and depends upon autotroph population, light conditions, and available gases etc.

Oxygen is considered to be a limiting factor, especially in lakes and in waters with a heavy load of organic material. Organisms have specific oxygen requirements. Low dissolved oxygen may prove fatal for many organisms for their survival.

The Dissolved oxygen in water can be determined by the following two methods :

1. Electrode or oxygen meter method, and
2. Iodometric method (Winkler's method)

Oxygen Meter Method: This method is convenient, quick and reasonably accurate.

Materials

1. Oxygen meter with dissolved oxygen probe.
2. Electrical Stirrer.
3. 5% sodium sulphite solution.

Method

Read the operation manual carefully and adjust the instrument accordingly. Dip the D.O. probe in 5% sodium sulphite solution with constant stirring. Set meter to zero mark. Now dip the D.O. probe in water sample being constantly stirred, and record the dissolved oxygen in mg/litre from the scale.

Winkler's Method

Principle: Oxygen combines with $Mn(OH)_2$ and forms higher hydroxides which, on subsequent acidification in the presence of iodide, liberate iodine in an amount equivalent to the original dissolved oxygen content of the sample. The I_2 is then determined by titration with $Na_2S_2O_3$.

Materials and Reagents

1. BOD bottles (100–300 mL).

2. Manganous sulphate solution: Dissolve 100 g of manganous sulphate in 200 mL of previously boiled distilled water and filter the solution.

3. Alkaline potassium iodide solution: Weigh 50 g of potassium iodide and 100 g of potassium hydroxide. Dissolve the chemicals in 200 mL of previously boiled distilled water.

4. Sodium thiosulphate solution (0.025 N) : Dissolve 6.205 g of sodium thiosulphate, in 1 litre previously boiled distilled water and add a pallet of NaOH as a preservative. Keep it in coloured bottle.

5. Starch Indicator: Dissolve 1 g starch in 100 mL warm distilled water and add a few drops of toluene as preservative.

6. Concentrated Sulphuric Acid : Sp. gravity 1.84

Method

1. Take a glass stoppered BOD bottle of known volume (100–300 mL) and fill it with sample avoiding any bubbling. No air should be trapped in bottle after the stopper is placed.

2. Open the bottle and pour in each 1 mL of manganous sulphate and alkaline potassium iodide solution using separate pipettes. If the volume of sample is over 200 mL, add 2 mL of each reagent instead of 1 mL.

3. A precipitate will appear. Place the stopper and shake the bottle thoroughly. Sample at this stage can be stored for a few days, if required.

7. Add 2 mL of sulphuric acid to dissolve the precipitate, shake thoroughly.

8. Transfer gently (avoiding bubbling) whole content, or a known part of it, in a conical flask. Put a few drops of starch indicator. Titrate against sodium thiosulphate solution and note the end point when initial blue colour disappears.

Calculation: (i) If whole content is used for titration

$$DO \text{ mg / L} = \frac{V_1 \, N \times 8 \times 1000}{V_2 - V_3}$$

(ii) If a fraction of the content is used for titration

$$DO \text{ mg / L} = \frac{V_1 \, N \times 8 \times 1000}{V_4 \left(V_2 - \frac{V_3}{V_2} \right)}$$

where, *D.O.* = Dissolved oxygen

V_1 = Volume of titrant (mL)

V_2 = Volume of sampling bottle after placing the stopper (in mL)

V_3 = Volume of manganous sulphate + potassium iodide added (mL)

V_4 = Volume of fraction of the content used for titration (mL)

N = Normality of titrant (0.025). The equivalent weight of oxygen is 8

Note : 1. To obtain the value of *DO* in mL/litre divide the *DO* in mg/litre by 1.43.

2. The original Winkler's method is modified by adding sodium azide in alkaline potassium iodide solution. This avoids the interference due to organic matter and chlorides present in the sample. The other reagents and procedure are the same as described above.

Preparation of Alkaline KI Solution For Modified Winkler's Method : Dissolve separately 350g KOH and 75 g KI in distilled water, mix the two and make the volume 500 mL with distilled water. Dissolve separately 5g of sodium azide (NaN_3) in 20 mL of distilled water. Mix alkaline iodide and sodium azide solutions.

BIOCHEMICAL OXYGEN DEMAND (BOD)

The Biochemical oxygen demand can be defined as amount of oxygen required by micro-organisms to stabilize biologically decomposable organic matter in the waste water under aerobic conditions. It is an approximate measure of the amount of biochemically degradable organic matter present in the sample. More the oxidizable organic matter present in water, greater the amount of oxygen required to degrade it biologically, hence more the BOD. The BOD test is generally performed to determine :

1. Degree of pollution in the lakes and streams at any given time.
2. Pollution load of waste water on the environment.
3. Efficiency of waste treatment plants etc.

CHEMCIAL OXYGEN DEMAND (COD)

The Chemical oxygen demand (COD) measures the amount of oxygen required for oxidation of organic compounds which are present in water by means of chemical reactions, involving oxidizing substances such as potassium dichromate and potassium permanganate. Potassium dichromate is the most suitable oxidant, but for water having more than 2g/l of Chlorides, potassium permanganate is used although the results are more variable because the latter is self-oxidizing.

TEST OF BIOCHEMICAL OXYGEN DEMAND (BOD)

The BOD can be evaluated by measuring oxygen concentration in a sample, idometrically before and after incubation in the dark at 20°C for 5 days. Preliminary dilution and aeration of sample (with the help of dilution water) are necessary to ensure that not all the oxygen is consumed during incubation. Excess dissolved oxygen must be present during the whole incubation. Samples absorbing more than 6 mg/L of oxygen should, therefore,

be diluted with a synthetic dilution water made from BOD free (distilled) water (reagent 4) to which the major constituents are added in the same concentration as in the sample. Sometimes a culture of bacteria (seed material) is also added, so that more of the organic matter is used up during the incubation. Generally, sewage is used as a standard seed material in the process.

Materials and Reagents

1. BOD incubator, BOD bottles

2. All reagents used in determination of dissolved oxygen as discussed in foregoing pages.

3. **BOD-free water :** Pass the deionized glass distilled water through a column of activated carbon and redistil it.

4. **Phosphatel buffer solution :** Dissolve 42.5 g potassium dihydrogen phosphate in 700 mL BOD free water and add 8.8 g NaOH. Adjust the pH at 7.2. Add 2 g ammonium sulphate and dilute to 1 litre with BOD feee water.

5. **Magnesium sulphate solution :** Dissolve 82.5 g of magnesium sulphate in BOD-free distilled water to prepare 1 litre of solution.

6. **Calcium chloride solution :** Dissolve 27.5 g of anhydrous calcium chloride in BOD-free distilled water to prepare 1 litre of solution.

7. **Ferric chloride solution :** Dissolve 0.25 g of ferric chloride in 1 litre of BOD-free distilled water.

8. **Sulphuric acid (1N) :** Add 2.8 mL of concentrated sulphuric acid to 100 mL of BOD-free distilled water.

9. **Sodium hydroxide solution (1N) :** Add 4 g of sodium hydroxide in BOD free distilled water and make the volume 100 mL.

10. **Allylthiourea solution :** Dissolve 500 mg of allylthiourea in distilled water and make the volume 1 litre.

Method

Preparation of Dilution Water

1. To prepare synthetic dilution water, aerate the required volume of BOD free distilled water in a glass container by bubbling compressed air for 1 to 2 days to attain dissolved oxygen saturation. After saturation it is kept at 20⊃C for atleast one day. Add per litre of this water 1 mL each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution and ferric chloride solution. If required, add requisite amount of seed (sewage) also.

2. **Dilution of sample:** Adjust the pH of sample to neutrality (around 7.0) using 1 N sulphuric acid or 1 N sodium hydroxide solution, as the case may be. To ensure that not all the oxygen of sample is exhausted during incubation, dilute the sample with appropriate amount of dilution water according to the expected BOD content of the sample.

Dilution of sample required for various ranges of expected BOD.

Expected BOD (mg/L) in sample	Volume of sample (mL)/L of mixture*	Dilution factor
0–6	1000	1
4–12	500	2
10–30	200	5
20–60	100	10

40–120	50	20
100–300	20	50
200–600	10	100
400–1200	5	200
1000–3000	2	500
2000–6000	1	1000
>6000	0.5	2000

***mixture denotes the diluted water sample.**

3. Fill two sets of BOD bottles of either 125, 250, or 300 mL capacity with this diluted water (sample) and add 1 mL of allylthiourea solution to each bottle. As far as possible avoid entrapping air bubbles in BOD bottles.

4. Stopper the bottle immediately.

5. Determine the dissolved oxygen content (D.O.) in one set immediately following the Winkler's method of oxygen estimation as described earlier. Incubate the other set in BOD incubator. Take out the bottles after 5 days and determine immediately their dissolved oxygen content (D_5).

Calculations :

$$\text{COD (mg/}L\text{)} = \frac{(B-T)\times N\times 1000\times 8}{\text{Volume of sample (mL)}}$$

where, D.O. = initial dissolved oxygen in the sample (mg/L); and D_5 = dissolved oxygen left out in the sample after 5 days Incubation (mg/L).

Note : In tropical and subtropical regions where rate of metabolism is relatively high an incubation for 3 days at 27°C (instead of 5 days at 20°C) is in practice and BOD so determined is expressed as BOD_3 mg/L.

TEST OF CHEMICAL OXYGEN DEMAND (COD)

Principle

Most of the organic matter decomposes and produces carbon dioxide and water when it is boiled with a mixture of potassium dichromate and sulphuric acid. A sample is refluxed with a known amount of potassium dichromate in the sulphuric acid medium and the excess of dichromate is titrated against ferrous ammonium sulphate (FAS). The amount of dichromate thus consumed is proportional to the oxygen required to oxidize the organic matter in the sample.

Materials and Reagents

1. COD reflux unit consisting of flat bottom flask with ground glass mouth (250 mL) and Leibig (straight tube, single surface) condenser (30 cm).

2. Hot water bath or heating mantle

3. Potassium dichromate solution (0.25 N): Dissolve 12.259 g of AR grade potassium dichromate, previously dried at 103⊃C in distilled water. Add about 120 mg sulphamic acid to this and dilute to 1 litre.

4. Dry powder of silver sulphate

5. Dry powder of mercuric sulphate

6. Concentrated sulphuric acid

7. **Ferroin indicator solution:** Dissolve 0.695 g of ferrous sulphate and 1.485 g of 1, 10-phenonthroline in distilled water to make 100 mL of indicator solution.

8. **Standard ferrous ammonium sulphate solution (0.25 N) :** Dissolve 98 g of ferrous ammonium sulphate in distilled water, add 20 mL of sulphuric acid, cool and dilute to 1 litre by further adding distilled water. To standardize this solution, dilute 25 mL of potassium dichromate solution to about 250 mL with distilled water, add 20 mL of sulphuric acid, and cool it. Add 5–6 drops of ferroin indicator solution and titrate against ferrous ammonium sulphate solution. The colour changes from blue green to a reddish blue at end point. The exact normality of FAS is calculated as given below :

$$\text{Normality of FAS} = \frac{\text{Volume of } K_2Cr_2O_7 \text{ (mL)} \times 0.25}{\text{Volume of FAS (mL)}}$$

Method

1. Take 20 mL of sample in the flask of reflux unit and add 10 mL of potassium dichromate solution, a pinch of each silver sulphate and mercuric sulphate, and 30 mL of sulphuric acid.

2. Attach Liebig condenser to the mouth of flask and heat the flask on a hot water bath or heating mantle for at least 2 hours to reflux the contents.

3. Cool the flask, detach from unit and dilute its contents to about 150 mL by adding distilled water.

4. Add 2–3 drops of ferroin indicator solution and titrate against ferrous ammonium sulphate solution. At the end point blue green colour of contents changes to reddish blue. Run simultaneously a distilled water blank in similar manner.

Calculations:

$$COD \text{ (mg / L)} = \frac{(B - T) \times N \times 1000 \times 8}{\text{Volume of sample (mL)}}$$

SEPARATION, DETECTION AND ESTIMATION OF HERBICIDES

The Chlorine substituted phenoxy acids and esters are commonly used as selective **herbicides** in agriculture, and as a result can give rise to residues in crops. Herbicides are also absorbed by the soil and river water. The method developed by Muller (1971) for the analysis of chlorophenoxy acids, requires formation of methyl ester followed by partition chromatography, separation and ultraviolet (UV) measurement. Gas liquid chromatographic (GLC) methods for the estimation of chlorophenoxyl acids are also suggested for the purpose.

High performance liquid chromatography (HPCL) method has been developed in the laboratory for the analysis of 2, 4–dichlorophenoxy acetic acid (2, 4–D), 4–(2, 4–dichlorophenoxy)–butyric acid (2, 4–DB); 2, 4, 5–trichlorophenoxy acetic acid), (2, 4, 5–T) and 2–(2, 4, 5–trichlorophenoxy propionic acid) (silvex) and for their corresponding methyl esters. The compounds are separated by using the gradient elution system of mobile phase and the eluate is monitored by a UV detector at 280 nm. Here calculations are based on peak height measurement of the sample and the standard for determining the concentration of species.

A variable wavelength ultraviolet monitor model Lamb–da–Max Model 481 fitted with a flow cell and set at 280 nm is used as a detector. For the sample injection, a universal liquid chromatograph injector model U6K stop flow injector is used. Samples are injected on to the stainless steel fine mesh

gauze fitted on top of the packing. Nuclear Magnetic Resonance. (N.M.R.) can also be used for detection and estimation of herbicides, insecticides, pesticides and disinfectants.

Reagents and Chemicals used in Analysis

Commercial grade insecticides etc. are used for chromatographic analysis. The following reagents can be used:

(i) Acetonitrile, (ii) Acetone, (iii) Acetonitrile, saturated with redistilled petroleum ether, (iv) 95 % Ethyl alcohol, (v) 2 % Alcoholic alkali solution, (vi) Acetone, (vii) Carbon–tetrachloride, (viii) Benzene, (ix) Celite 545, (x) Chloroform, (xi) Charcoal, (xii) Chromium trioxide, (xiii) Dichlorodimethyl saline, (xiv) Eluting trioxide, (xv) Eluting solvents 5 %, 15 % and 60 % prepared by dissolving 60 mL, 160 mL and 500 mL ethylether to 1 litre petroleum ether, (xvi) Ethylacetate redistilled, (xvii) Ethyl ether, (xviii) Florisil, AR grade, (xix) Glacial acetic acid BDH, (xx) Hexane, (xxi) Isopropyl alcohol, (xxii) Magnesia, prepared as follows: slurry about 500 gm of magnesium oxide with water, heat on steam bath for 30 minutes and filter with suction. Dry overnight at 115 – 130 ⊃ C and pulverize to pass No. 60 sieve. Store in closed container, (xxiii) Petroleum ether, (xxiv) Sea sand, (xxv) Silica gel, chromatographic grade, (xxvi) Sodium sulphate, anhydrous, (xxvii) Sulphuric acid, sp. gr. 1.84 (xxviii) 2, 2, 2, Trimethylpentane (ISOOCTANE), (xxix) Adsorbent; Charocal or Aluminium Oxides, (xxx) Chromogenic reagents : 0.2% $AgNO_3$.

Experimental Technique

One litre of sample is quantitatively transferred into a two litre separatory funnel and acidified to pH 2 (approx) with sulphuric acid. pH is checked with an indicator paper. Water sample is then partitioned with 60 mL of acetone by shaking for 2 min. Sample is then extracted with 60 mL of dichloromethane and hexane (1 : 1) by shaking for 2 minutes and kept for some time. Water layer is collected in the original water sample container and organic phase is collected in a 500 mL Kudema–Danish flask. The extraction is then repeated twice with 50 mL each of dichloromethane and the solvent treated as above. Solvent extract volume is then reduced to 0.5 mL to 0.6 mL under diminished pressure. Few microlitres of this concentrate is then injected to HPLC for the analysis of chlorophenoxy acids.

Determination of Pesticides

For analysis of pesticides like BHC, aldrin, dieldrin, heptachlor, *p, p′*,–DDT, *p, p′*,–DDE and p, *p′*–DDD in water, the sample is collected in stainless steel container of 500 mL capacity to which a nylon chord is tied. The whole assembly is lowered below the water surface, raised two to three times and then withdrawn, and water is then transfered into precleaned amber coloured bottle. One litre of water is collected for the analysis of pesticide residues. The water is then filtered and extracted on the same day of collection and after that ten fold concentrations are subjected to gas chromatographic analysis. The detection and estimation can be done by nuclear magnetic resonance (n.m.r.)

Gas Chromatographic Conditions

A stainless steel column, $6\,\text{ft} \times \frac{1}{8}$ inch packed with 3% or 101 on chromosorb W–HP (100–200) is employed for the analysis in chlorinated

hydrocarbon pesticide mixture. Temperature is programmed from 170°C for 26 minute to 200°C for 10 minutes at the rate of 15°C/minute). Injector and detector are kept at 225°C. Flow rate of carrier gas nitrogen is maintained at 25 mL/minute.

WATER POLLUTION LAWS

There are two types of enactments about water pollution in India:

1. Central Enactments
2. State Enactments

1. Central Enactments : Acts, which are passed by Government of India, are given below :

(a) North India Canal and Drainage Act, (1873)

(b) Indian Fisheries Act, (1897)

(c) The Damodar Valley–Corporation (Prevention of Pollution of Water) Regulation Act (1948)

(d) The River Boards Act, (1956)

(e) The Water (Prevention and Control of Pollution) Act (1974), Amended in 1988.

(f) The water (Prevention and Control of Pollution) Cess Act, (1977)

(g) The Environment (Protection) Act (1986)

(h) The Merchant Shipping (Amendment) Act, (1987)

2. State Enactment: Acts, which are passed by State Governments are called state enactments. These laws, about water pollution, are given below:

(a) The Orissa River Pollution Prevention Act, (1953)

(b) The Maharashtra Prevention of Water Pollution Act, (1969)

WATER QUALITY RELATED PROBLEMS IN PUBLIC HEALTH, ENVIRONMENT AND AGRICULTURE

The knowledge of water quality and its contents is very essential for judging its suitability for different purposes such as drinking, irrigation, industry, public health and environmental safety.

There are various types of standards in view of the consumption of water like:

1. Drinking water standards
2. Bacteriological standards
3. Irrigation standards
4. Stream standards
5. Effluent standards

1. Drinking Water Standards: Water is directly consumed by all human beings. There are certain national as well as international authorities, who have laid down various standards for domestic use of drinking water. For this purpose the main Indian agencies are like Indian Council of Medical Research (ICMR), Bureau of Indian Standards (BIS), and Ministry of Works and Housing (MWH) whereas international agency is named as World Health Organisation (WHO). Some important drinking water standards are as follows:

Drinking Water Standards

Characteristics	World Health Organization (WHO)		Ministry of Works and Housing (1975)	
	Highest desirable	Maximum permissible	Acceptable	Causes of rejection
Physico-chemical				
Turbidity (J.T.U.)	5.0	25.0	2.5	10.0
Colour (Pt-scale)	5.0	50.0	5.0	25.0
Taste and Odour	Nothing	disagreeable	unobjectionable	
pH	7.0–8.5	6.5–9.2	7.0–8.5	6.5–9.2
Total solids	500	1500	500	1500
Total hardness	100	500	200	600
Chlorides	200	600	200	1000
Sulphates (as SO_4)	200	400	200	400
Fluorides (as F)	1.0	1.5	1.0	1.5
Nitrates (as NO_3)	45	45	45	45
Calcium (as Ca)	75	200	75	200
Magnesium	30	150	30	150
Iron (as Fe)	0.1	1.0	0.1	1.0
Manganese (as Mn)	0.05	0.5	0.05	0.5
Copper	0.05	1.0	0.05	1.5
Zinc	5.0	15.0	5.0	15.0
Phenolic compounds	0.001	0.002	0.001	0.002
Detergents, anionic	0.2	1.0	0.2	1.0
Mineral oil	0.01	0.30	0.01	0.30
Arsenic	0.05	0.05	0.05	0.05
Chromium (as Cr^{6+})	–	0.01	0.05	0.05
Cyanide	–	0.05	0.05	0.05
Lead	–	0.10	0.10	0.10
Selenium	–	0.01	0.01	0.01
Cadmium	–	0.01	0.01	0.01
Mercury	–	0.001	0.001	0.001
PCBs (µg/L)	–	0.2	0.2	0.2
Gross alfa-activity (PCi/L)	–	3.0	3.0	3.0
Gross beta-activity (PCi/L)	–	30.0	30.0	30.0

2. Bacteriological Standards: Bacteriological standards in water are standardized by World Health Organisation and Ministry of Works and Housing, both such as:

(A) By WHO

(a) Water Entering Distribution System: Coliform bacteria Count in any sample of 100 mL should be zero.

(b) Water in Distribution System: All samples taken from the distribution system including consumer's premises should be free from coliform organisms. Since in practice it is not always possible, even then following standards can be followed:

(i) Throughout the year, 95% of the samples examined should not have any coliform organisms.

(ii) E.coli count in any 100 mL sample should be zero.

(iii) Coliform organisms not more than 10/100 mL should be present in any sample.

(iv) Coliform organisms should not be detectable in 100 mL of any two consecutive samples.

(B) Ministry of Works and Housing

(a) Water Entering Distribution System: Coliform count in any sample of 100 mL should be zero.

(b) Water in Distribution System: shall be satisfied by all the three criteria given below:

(i) E–coli count in 100 mL of any sample should be zero.

(ii) Coliform organisms not more than 10/100 mL should be present in any sample.

(iii) Coliform organisms should not be detectable in 100 mL of any two consecutive samples of more than 50% of the samples collected for the year.

3. Irrigation Standards: The quality of water for irrigation purpose should be up to the mark. The major parameters of concern are:

(a) Salinity

(b) Water infiltration camp

(c) Specific Ion Toxicity

(d) Miscellaneous

(e) Sodium concentration

(a) Salinity: The salinity of water should be less for irrigation purpose, because salts in water reduce water availability to the crops to such an extent that yield is affected. Salinity of water is denoted by dissolved solids and conductivity.

(b) Water Infiltration Rate: High sodium and low calcium content of water reduces the rate at which irrigation water enters soil to such an extent that sufficient water can not be infiltrated to supply the crop adequately from one irrigation to the next.

(c) Specific Ion Toxicity: The presence of some ions such as sodium, boron or chloride in irrigation water accumulates in crops. It causes crop damage and reduces yield, if the ion concentration is considerably high.

(d) Miscellaneous: When nutrients in water are in excess, it causes reduction in yield. These nutrients deposit on fruits or foliage.

(e) Sodium Concentration: Sodium in water can be denoted by:

(i) Sodium, (ii) Sodium absorption ratio (SAR) and (iii) Residual Sodium Carbonate (RSC).

These parameters in water can be calculated by the following formulae and the values of individual constituents are taken in mEq./L.

$$\text{Percent Sodium} = \frac{Na^+}{Ca^{2+} + Mg^{2+} + K^+ + Na^+} \times 100$$

$$SAR = \frac{Na^+}{\frac{Ca^{2+}\ Mg^{2+}}{2}}$$

$$RSC = (CO_3 + HCO_3) - (Ca^{++} + Mg^{++})$$

Suitability of water with different constituents (Total dissolved solids, sulphate, chloride, sodium, boron) for irrigation purpose is given below:

Class of water	TDS ppm	Sulphate ppm	Chloride ppm	% sodium	Boron ppm	E.C. mS/cm	Suitability for irrigation
I	0–700	0–192	0–142	0–60	0–0.5	0–750	Excellent to good for irrigation
II	700–2000	192–480	142–355	60–75	0.5–2.0	750–2250	Good to injurious, suitable with permeable soil and moderate leaching, harmful to sensitive crops
III	>2000	>480	>355	>75	>2.0	>2250	Unfit for irrigation

Suitability of water for irrigation with different values of sodium absorption ratio (SAR) is given below:

SAR	Water-Suitability for irrigation
0–10	Suitable for all types of crops and all types of soils except for those crops which are highly sensitive to sodium
10–18	Suitable for coarse textured or organic soil with good permeability. Relatively unsuitable in fine textured soil.
18–26	Harmful for almost all types of soils. Requires good drainage, high leaching and gypsum addition.
>26	Unsuitable for irrigation

Trace elements are also present in irrigation water. Their limit should be as given below:

Trace Elements	Limit (mg/L)
Aluminium	1.0
Arsenic	1.0
Boron	0.75
Cadmium	0.005
Chromium	5.0
Cobalt	0.2
Copper	0.2
Lead	5.0
Manganese	2.0
Molybdenum	0.005
Nickel	0.5
Selenium	0.05
Zinc	5.0

4. Stream Standards: For each typical use of water (like irrigation, drinking, industry, power generation, recreation etc.), water quality criteria are established. Any water body can be designed for some particular best use which can be termed as 'designated best use'.

On the basis of the designated best use of water, water resources can be classified and zoned in different categories. For fresh water, the water quality criteria are classified by Central Pollution Control Board (CPCB) and State Pollution Control Boards (SPCB). The above named boards have adopted a scheme of classification and zoning of water bodies, which is given below:

Classification and zoning of water bodies (CPCB 1979)

Designated-best-use	Nomenclature for the class of water
Fresh water	
Drinking water source without conventional treatment but after disinfection.	Class *A*
Out-door bathing	Class *B*
Drinking water source with conventional treatment followed by disinfection	Class *C*
Propagation of wildlife, fisheries	Class *D*
Irrigation, industrial cooling, and controlled waste disposal.	Class *E*
Sea waters (Including Estuaries and Coastal waters)	
Salt pans, shell fishing, contact sport	SW I
Commercial fishing, non-contact recreation	SW II
Industrial cooling	SW III
Harbour	SW IV
Navigation, controlled waste disposal	SW V

In above data, 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.

Water quality criteria for fresh water classification (CPCB, 1979)

Classes*	Criteria
Class A	Dissolved oxygen (minimum 6 mg/L), BOD (maximum 2 mg/L), MPN of coliforms per 100 mL (maximum 50), pH (6.5–8.5)
Class B	Dissolved oxygen (minimum 5 mg/L), BOD (maximum 3 mg/L), MPN of coliform per 100 mL (maximum 500), pH (6.5–8.5)
Class C	Dissolved oxygen (minimum 4 mg/L), BOD (maximum 3 mg/L), MPN of coliforms per 100 mL (maximum 5000), pH (6.0–9.0)
Class D	Dissolved oxygen (minimum 4 mg/L), pH (6.5–8.5), Free ammonia as N (Maximum 1.2 mg/L)
Class E	pH (6.0–8.5), Electrical conductivity (maximum mS/cm 2,250), Sodium absorption ratio. SAR (maximum 26), Boron (maximum 2 mg/L)

5. Effluent Standards : The standards of effluent are related to the quality of waste waters, which are originated from industry, community, and agriculture. Some important effluent standards in land surface water, public sewers, land for irrigation and marine coastal area were fixed by Central Pollution Control Board in 1995. These data are given as follows:

General standards for discharge of effluents (CPCB, 1995)

Parameter		Standards			
		Inland surface water (a)	Public sewers (b)	Land for irrigation (c)	Marine coastal area (d)
1.	Colour and odour				
2.	Suspended solids, mg/L, Max.	100	600	200	(a) For process waste water-100 (b) For cooling water effluent10 per cent above total suspended matter of influent cooling water
3.	Particle size of suspended solids	Shall pass 850 micron IS sieve	–	–	(a) Floatable solids, Max 3 mm (b) Settable solids max 850 microns
4.	Dissolved solids (inorganic), mg/L max	2100	2100	2100	–
5.	pH value	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0
6.	Temperature ⊃C, max	Shall not exceed 40 in any section of the stream within 15 metres down stream from the effluent outlet	45 at the point of discharge	–	45 at the point of discharge
7.	Oil and grease mg/L max	10	20	10	20
8.	Total residual chlorine mg/L max	1.0	–	–	1.0
9.	Residual sodium carbonate, mg/L max.	–	–	5.0	–
10.	Cyanide (as CN), mg/L Max.	0.2	2.0	0.2	0.2
11.	Chloride (as Cl), mg/L Max.	1000	1000	600	–
12.	Fluoride (as F), mg/L Max.	2.0	15	–	15
13.	Dissolved Phosphates (as P), mg/L Max.	5.0	–	–	–
14.	Sulphate (as SO_4), mg/L Max.	1000	1000	1000	–
15.	Sulphide (as S), mg/L Max.	2.0	–	–	5.0
16.	Pesticides	Absent	Absent	Absent	Absent
17.	Phenolic compounds (as C_6H_5OH) mg/L Max.	1.0	5.0	–	5.0
18.	Radioactive materials Alpha emitters μC/mL, Max.	10^{-7}	10^{-7}	10^{-8}	10^{-7}
19.	Beta emitters μC/mL, Max.	10^{-6}	10^{-6}	10^{-7}	10^{-6}

Parameter		Standards			
		Inland surface water (a)	Public sewers (b)	Land for irrigation (c)	Marine coastal area (d)
20.	Percent Na	–	60	60	–
21.	Ammonical Nitrogen (as N), mg/L Max.	50	50	–	50
22.	Total Kjeldahl nitrogen (as N), mg/L Max.	100	–	–	100
23.	Free Ammonia (as NH_3), mg/L Max.	5.0	–	–	5.0
24.	Biochemical oxygen demand (5 days at 20 ⊃ C Max.)	30	350	100	100
25.	Chemical oxygen demand, mg/L Max.	250	–	–	250
26.	Arsenic (as As), mg/L Max.	0.2	0.2	0.2	0.2
27.	Mercury (as Hg), mg/L Max.	0.01	0.01	–	0.01
28.	Lead (as Pb), mg/L Max.	0.1	1.0	–	1.0
29.	Cadmium (as Cd), mg/L Max.	2.0	1.0	–	2.0
30.	Hexavalent chromium (as Cr^{6+}), mg/L Max.	0.1	2.0	–	1.0
31.	Total chromium (as Cr), mg/L Max.	2.0	2.0	–	2.0
32.	Copper (as Cu), mg/L Max.	3.0	3.0	–	3.0
33.	Zinc (as Zn), mg/L Max.	5.0	15	–	15
34.	Selenium (as Se), mg/L Max.	0.05	0.05	–	0.05
35.	Nickel (as Ni), mg/L Max.	3.0	3.0	–	5.0
36.	Boron (as B), mg/L Max.	2.0	2.0	2.0	–

QUESTIONS

1. What do you understand by water pollution ? Classify the types of water pollution.
2. What is the origin of waste water ? Describe the water pollutants and their effects.
3. What are the sources of water pollution ? Give details about industrial wastes as source of pollution.
4. How are the pulp and paper industry, distillery, tanning industry and cane sugar industry responsible for water pollution ?
5. How do the pesticides and radioactive wastes pollute drinking water ?
6. How will you explain the physical, inorganic and organic water pollution ?

7. What are the objectives and parameters of water analysis ?
8. Describe the presence of colour, turbidity, total dissolved solids, hardness and acidity in a water sample.
9. What are the different forms of nitrogen ? Describe the phenol disulphonic acid method of analysis of nitrate ions in water.
10. Give the difference of analysis between:
    (a) Acidity and akalinity of water
    (b) Chloride and fluoride in water
    (c) Turbidity and conductivity of water
    (d) Sulphates and phosphates of water
11. Describe the Platinum–Cobalt method of colour analysis in a water sample.
12. What do you know about conductivity of water ? How will you determine it in a water sample ?
13. What is the importance of silica in water ? Describe its presence in a water sample.
14. How will you determine the presence of ammonia and nitrite in water ?
15. What is dissolved oxygen in water ? Describe a suitable method to determine it.
16. How will you calculate the dissolved oxygen in water by Winkler's method ?
17. What is the difference between biological oxygen demand and chemical oxygen demand of water ?
18. How can the BOD be evaluated in a water sample ?
19. Describe the method of determination of chemical oxygen demand in water.
20. How will you separate, detect and estimate the herbicide in a water sample ?
21. What are the standard conditions to determine the pesticides in water by gas chromatography.
22. What do you understand by heavy metal pollution ? How do cadmium, mercury, arsenic, and chromium pollute water ?
23. What is the public health significance of copper, lead, manganese and zinc metals ?
24. Determine the presence of heavy metals in water by atomic absorption spectrophotometer.
25. How will you measure iron and manganese in a water sample ?
26. Describe a suitable method to determine zinc and copper metal in water.
27. Give a complete method to estimate Cd, Cr, Ni and Pb in a water sample.
28. What are drinking water standards ? Give the laws of water pollution.
29. Classify water standards and describe the bacteriological and irrigation standards of water.

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CHAPTER

3

ANALYSIS OF FOOD PRODUCTS

INTRODUCTION

Food analysis is that branch of chemistry which deals with the study of analytical procedures, development, applications, characterisation of the properties of foods and their constituents. These analytical procedures are used to characterize the different properties of foods like their composition, physiochemical properties, structure, and sensory attributes. This information provides an idea to the consumers about a safe, nutritious, desirable food and also an ability to economically produce foods, suitable for their diet.

The objective of this chapter is to review the basic principles of the analytical procedures commonly used to analyze foods.

REASONS FOR ANALYZING FOOD

Foods are analyzed by scientists, who are working in major sectors of the food industry including ingredient suppliers, laboratories of analytical work, Government Laboratories, food manufacturers, and University research laboratories. There are various purposes for analyzing the foods, which are as follows :

1. Government Regulations and Recommendations
2. Standards
3. Nutritional Labeling
4. Authenticity
5. Food inspection and grading

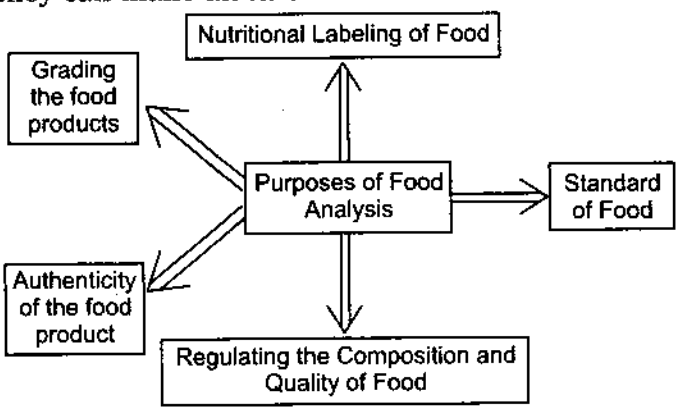
1. Government Regulations and Recommendations

Government regulations and recommendations are designed to maintain the

(a) General quality of the food supply.

(b) To ensure the consumers about the food products which are provided by industries that the quality of food is safe and wholesome.

(c) To give information to consumers about the nutritional composition of foods so that they can make an informed choice of their diet.



(d) To enable healthy and fair competition among food industries.

(e) To eliminate economic fraud.

There are various departments which are responsible for regulating the chemical composition and quality of foods like Agmark, FPO etc.

2. Standards

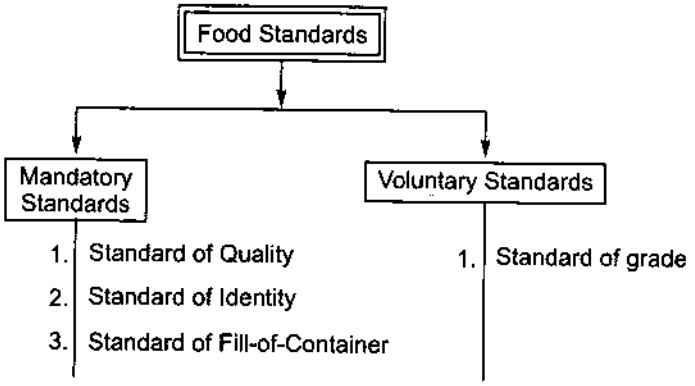
Government agencies have specified a number of standards which depend on the

- ❖ Composition
- ❖ Quality
- ❖ Inspection
- ❖ And labeling of specific food products

There are following types of food standards:

(a) Mandatory

(b) Voluntary



(a) Mandatory Standards : The mandatory standards are:

(i) Standard of Quality : Standards of quality are defined for canned fruits and vegetables etc. The quality of food is concerned with minimum requirements of the colour, tenderness, mass and freedom from defects.

(ii) Standards of Identity: These regulations are concerned with the type and amount of ingredients that certain foods must contain if these are to be called by a particular name on the food label. For some foods there is a maximum or minimum concentration of a certain component that they must contain, e.g., "peanut butter" must have less than 55% fat, "ice cream" must have greater than 10% milk fat, "cheddar cheese" must possess more than 50% milk fat and less than 39% moisture.

(iii) Standards of fill-of-Container: These standards of food state how the degree of fill is measured as well as specify how full a container must be to avoid consumer deception.

(b) Voluntary Standards

Standard of Grade : The grading of food products depends upon their quality and is stated as 'standard grade' or 'excellent grade'. A number of foods, including meat, dairy products and eggs are graded according to their quality.

Specification of the grade of a food product on the label is voluntary, but many food manufacturers opt to do this because superior grade products can be sold on a higher price. The Government has set up laboratories to which the manufacturers send their products to be tested and to receive the appropriate certification. This service is requested and paid for by the food producers.

3. Nutritional Labeling

The Governments have passed various regulations pertaining to the nutritional labeling of foods, and making it mandatory for almost all food products to have standardized nutritional labels. These regulations provide the choice to consumers about their diet. One of the major reasons for introducing .this nutritional labeling is to state the total calorific value of the food. as well as total fat, saturated fat, cholesterol, sodium, carbohydrate, sugars, protein, dietary fiber, vitamins, iron and calcium. The label may also contain information about nutrient content claims (such as "low fat", "low sodium", highfiber", "fat free" etc.). The label may also contain some approved health claims based on links between specific food components and certain diseases *e.g.*, calcium and osteoporosis, sodium and high blood pressure, soluble fibre and heart disease and cholesterol and heart disease. The information provided on the label can be used by consumers to plan a nutritious and balanced diet, to avoid over consumption of food components and ingredients which are linked with health problems and to encourage consumption of foods that are beneficial to health.

4. Authenticity

The quality of food ingredients dictates the price of certain foods.

EXAMPLES

(a) A packet of premium coffee may claim that the coffee beans are imported from Columbia.

(b) The label of an expensive wine may claim that it was produced in a certain region; using a particular type of grapes in a particular year.

There are many instances in the past years when manufacturers had made false claims about the authenticity of their products in order to get a higher price. Hence it is important to have an analytical technique which can be used to test the authenticity of certain food components, to ensure that consumers are not the victims of economic fraud and that competition among food manufacturers is fair.

5. Food Inspection and Grading

For routine analysis of the properties of food products, government has Food Inspection and Grading laboratories. Both government and food manufacturers need analytical techniques which provide the appropriate information about food properties. For this type of test the most important criterion is often the accuracy of the measurements and the use of an official method. The government has carried out a survey of many official analytical techniques developed to analyze the foods, and has specified the techniques which must be used to analyze a certain food component for labeling purposes. The technique is simple, inexpensive to perform, accurate and reliable, should be chosen for analysis of a food product.

FOOD SAFETY

For analyzing the foods, one of the most important reasons from both the consumer and the manufacturer standpoint is to ensure that they are safe. If a food manufacturer sells a product which is harmful and toxic, it would be economically disastrous as well as unpleasant to the consumer. If a food contains harmful microorganisms like Listeria and Salmonella,or toxic chemicals (*e.g.*, pesticides, herbicides)and extraneous matter (*e.g.*, glass, wood, metals, insect matter), it is unsafe. Therefore food manufacturers do

everything they can to ensure that these harmful substances are not present or that they are eliminated before the food is consumed. Hence it is important to use analytical techniques which have a high sensitivity; *i.e.*, which can reliably detect even low levels of harmful substances. Food manufacturers and government laboratories routinely analyse food products to ensure and confirm the absence of harmful materials and whether the food production process is operating correctly or not.

ANALYSIS OF MOISTURE OF FOOD-MATERIALS

1. MOISTURE

This is the water content, which may be present in various food materials, and can be analysed by the following procedures. We are taking samples of vegetable oils, spices, butter and honey to analyse the moisture:

Moisture in Vegetable Oils : For the determination of moisture in vegetable oils, method is described here:

HOT-PLATE METHOD

Apparatus

1. Glass beaker — 100–150 ml capacity
2. Small glass rod — one
3. Desiccator — containing an efficient desiccant such as phosphorus pentaoxide.
4. Electric hot-plate with variable heat control.

Procedure

1. Weigh accurately 10 gm of the oil or fat in a previously dried glass beaker and keep in a Desiccator.
2. Heat the sample on the electric hot-plate, stir continuously with the glass rod.
3. Avoid sputtering of oil or fat during heat.
4. Stop heating when bubbles of steam and foam are absent. This is the end point. (End point can be judged by placing a clean and dry watch glass on top of the beaker and observe when no further condensation takes place on the watch glass).
5. Cool in the desiccator and weigh it.

Calculations: Moisture and volatile Content $= \frac{W_1}{W_2} \times 100$

Where,

W_1 = Weight of the material after heating (in gm).

W_2 = Weight of the material taken for the test (in gm).

Note: Excess value of moisture present over the standard value (*i.e.*, 0.1-1.0%) indicates the moisture contamination.

Moisture in Spices and Condiments

Apparatus

1. Dean and Stark apparatus
2. Hot plate.

Reagents required:

1. Toluene B.P = 111°C (redistilled)

Procedure

1. Weigh accurately 25 gm of the well ground sample.

2. Transfer the sample into the moisture distillation flask. (Flask and the trap should by dried thoroughly before use).

3. Set up the trap with distillation flask and apply vaseline at the joints of apparatus. Pour the toluene through the trap.

4. Now heat the flask on a hot plate under reflux condenser for about 4 hours until the water drops are not collected in the trap.

5. Measure the volume of water collected in the trap and calculate the percentage of moisture.

Calculations: % of Moisture (by weight) $= V \times \frac{\text{sp. gravity}}{W} \times 100$

where, V = Volume of water collected in the trap

Sp gravity = Specific gravity of water at the temperature at which the volume of water is read.

W = Weight of the sample taken for test.

Note : Excess value of moisture present over the standard value (*i.e.*, 10–12.0%) indicates the moisture contamination.

Moisture in Butter and Ghee

Apparatus

1. Electrical Air-oven with temperature control
2. Aluminium flat bottom dishes of diameter 2" depth 3/4", width 0.012".
3. Desiccator

Preparation of sample: Soften the sample with gentle heat (if hard), but do not melt, mix the sample thoroughly to distribute the moisture.

Procedure

(a) Weigh accurately 10 gm of prepared sample in a tarred moisture dish and keep in a desiccator.

(c) Put it in an air-oven at 105°C ± 2°C for 1/2 an hour

(d) Remove it from the oven and cool in a desiccator, and weigh it.

(e) Again keep in the oven for 1/2 an hour, cool in desiccator and weigh it.

(f) Repeat (d) until the loss of weight between two consecutive weighings does not exceed 1 mg.

(g) Note the final weight.

Calculations:% Moisture $= \frac{(W_1 - W_2)}{W_1} \times 100$

where, W_1 = Weight of the sample taken for test

W_2 = Weight of the sample after heating (final weight).

Note: Presence of excess value of moisture over the standard norm (of less than 0.2%) will indicate moisture contamination.

Moisture in Honey

Refractometer Method : Moisture of honey is determined by this method.

Procedure : Derermine the reading of honey (refractive index) by refractometer at 20°C and Calculate the percent moisture from the value given in the table.

Relationship between Refractive Index and Moisture Content of Honey

R.I. at 20°C	Moisture by wt. %	R.I. at 20°C	Moisture by wt. %	R.I. at 20°C	Moisture by wt. %
1.5044	13.0	1.4940	17.0	1.4840	21.0
1.5038	13.2	1.4935	17.2	1.4835	21.2
1.5033	13.4	1.4930	17.4	1.4830	21.4
1.5028	13.6	1.4925	17.6	1.4825	21.6
1.5023	13.8	1.4920	17.8	1.4820	21.8
1.5020	14.0	1.4915	18.0	1.4815	22.0
1.5012	14.2	1.4910	18.2	1.4810	22.2
1.5007	14.4	1.4905	18.4	1.4805	22.4
1.5002	14.6	1.4900	18.6	1.4800	22.6
1.4997	14.8	1.4895	18.8	1.4795	22.8
1.4992	15.0	1.4890	19.0	1.4790	23.0
1.4987	15.2	1.4885	19.2	1.4785	23.2
1.4982	15.4	1.4880	19.4	1.4780	23.4
1.4976	15.6	1.4875	19.6	1.4775	23.6
1.4971	15.8	1.4870	19.8	1.4770	23.8
1.4966	16.0	1.4865	20.0	1.4765	24.0
1.4961	16.2	1.4860	20.2	1.4760	24.2
1.4956	16.4	1.4855	20.4	1.4755	24.4
1.4951	16.6	1.4850	20.6	1.4750	24.6
1.4946	16.8	1.4845	20.8	1.4745	24.8
				1.4740	25.0

Note : The above table will show the moisture contamination in the sample. Moisture value more than standard value (*i.e.*, 25.0%) indicates contamination.

ASH

Ash is found as a residue after the burning of many food materials. This can be determined by different procedures. Here we shall analyse Ash in spices and honey.

ANALYSIS OF ASH

Total Ash of Spices

Apparatus

1. Silica crucible
3. Muffle furnace
4. Desiccator.
5. Balance

Chemicals required:

1. Absolute Alcohol
2. Hot water.

Procedure

1. Weigh accurately 2 gm ground material in a previously weighed silica crucible.

2. Spill about 2 ml absolute alcohol on the material and ignite it in closed muffle furnace at 550⊃C for 30 minutes.

3. Cool and break up the ash with hot water drops.

4. If wetting shows the ash with hot water to be carbon-free, place the crucible in desiccator and allow it to cool upto room temperature and weigh.

5. If the wetting shows carbon, repeat wetting and heating until no trace of carbon is visible. Now ignite it in a muffle furnace for 30 minutes after the disappearance of carbon.

Note : Reserve the ash for the determination of acid-insoluble ash, and water-insoluble ash.

Calculations : Total ash (% by weight) $= \frac{(W_2 - W)}{(W_1 - W)} \times 100$

where, W = Weight (in gm) of the empty crucible (in gm).

W_1 = Weight of the crucible with material taken for the test (in gm).

W_2 = Weight of the crucible with total ash (in gm).

Note : Standard value of total ash for spices should not exceed 7–9%.

ACID-INSOLUBLE ASH IN SPICES

Chemicals required: 1. Dilute hydrochloric acid: It is prepared by taking 2 ml conc. HCl diluted with 5 volumes of distilled water.

Procedure

1. Add 25 mL of dilute HCl to the silica crucible containing reserved total ash and boil it for 10 minutes, covering with watch glass to prevent sputtering.
2. Allow to cool and filter the contents through a quantitative filter paper.
3. Wash the filter paper with distilled water until the washings are free from acid (check with blue litmus paper) and retain it in the crucible.
4. Now evaporate the above content carefully on a steam-bath and ignite in the muffle furnace at 550°C for 1 hour.
5. Cool the crucible in a desiccator and weigh.
6. Repeat the process of igniting for 30 minutes, cooling and weighing till the difference in weight between two successive weighings is less than 1 mg.
7. Note the weight.

Calculations: Acid Insoluble Ash (% by weight) $= \frac{(W_2 - W)}{(W_1 - W)} \times 100$

where, W_2 = Weight of the crucible with the acid insoluble ash (in gm).

W = Weight of the crucible (in gm)

W_1 = Weight of the crucible with the material taken for the test (in gm).

Note : W and W_1 having the connections as total ash analysis.

Note : Excess value of acid-insoluble ash present over the standard value (*i.e.*, 1.0 to 1.5 %) indicates contamination.

WATER-INSOLUBLE ASH

The ash of food materials, which can not be dissolved in water, is analysed in the following way:

Chemicals required

1. Distilled Water
2. Ashless filter paper

Apparatus

1. Desiccator

2. Dish: Flat-bottomed platinum dish of 15 cm^2 surface area.

3. Muffle Furnace.

Procedure

1. To the reserved ash (as done in total ash procedure) in the dish, add distilled water, heat nearly to boiling, and filter through an ashless filter paper.

2. Wash the filter paper with hot water until the combined filtrate and washings measure about 60 mL.

3. Return the filter paper and contents to the dish, evaporate the water carefully on a water bath and ignite at 550°C ± 25°C for 1 hour.

4. Cool in the desiccator and weigh.

5. Ignite again, cool and weigh.

6. Repeat the process of igniting, cooling and weighing till the difference in mass between two successive weighings is less than 0.001 g.

7. Note the lowest mass.

Calculation:

$$\text{Water Insoluble ash (on dry basis)} = (M_2 - M_0) \times \frac{100}{M_1 - M_0} \times \frac{100}{100 - H}$$

where, M_2 = Mass in g of the dish and water insoluble ash.

M_1 = Mass in g of the dish and test portion

M_0 = Mass in g of the empty dish

H = Moisture content of the sample as received in percent.

Note : Variation in the value of residue ash from the standard norm indicates the level of contamination in the sample.

ASH OF HONEY

Apparatus

1. Pocelain dish or silica dish or platinum dish.
2. Dish
3. Hot-plate
4. Muffle furnace
5. Desiccator

Chemical Required

1. Olive oil

Procedure

1. Weigh accurately 2 gm of the honey sample in porcelain, silica or platinum dish and add a few drops of olive oil to prevent sputtering.

2. Heat it on a hot plate at low temperature until swelling ceases.

3. Ignite in a muffle furnace at 600°C till white ash is obtained.

4. Cool the dish in a desiccator and weigh.

Calculation: Ash (% by weight) $= \dfrac{(W_2 - W)}{(W_1 - W)} \times 100$

where,

W = Weight in gm. of the empty dish

W_1 = Weight in gm. of the dish with honey sample taken for test

W_2 = Weight in gm. of the dish with ash.

Note : Variation in the value of ash from the standard value (*i.e.*, more than 0.5%) indicates the level of contamination in the sample.

CRUDE FIBRES

Crude fibres are found in spices and condiments as common contaminants. We will see here how to analyse them.

ANALYSIS OF CRUDE FIBRES (OF SPICES)

Apparatus

1. Wide mouth conical Flask – 1000 ml and 500 ml capacity.
2. Beakers – 500 ml capacity.
3. Fine linen cloth – about 18 threads in a centimeter.
4. Gooch crucible with asbestos.
5. Hot plate.
6. Air-oven (electric).

Reagents required

1. Dilute sulphuric acid 1.25% (W/V).
2. Sodium hydroxide solution 1.25% (W/V).
3. Ethyl alcohol 95% by volume.
4. Ether

Procedure

1. Transfer the material to a one-litre flask and add ether to remove fat, and decant the ether.

2. Take 200 mL of 1.25% (W/V) H_2SO_4 in a beaker and boil it.

3. Transfer the boiling acid to the flask containing the fat-free material and immediately connect the flask with a reflux condenser and heat it to boil the contents for 30 minutes.

4. Stop heating and remove the flask, filter the contents through linen cloth.

5. Wash the residue with boiling water until the washings are free from acid (Check with blue litmus paper).

6. Bring same quantity of sodium hydroxide solution to the boiling under a reflux condenser.

7. Wash the residue through the filter paper, with 200 mL of sodium hydroxide solution.

8. Immediately connect the flask with the reflux condenser and boil for 30 minutes.

9. Remove the flask and immediately filter through the linen cloth.

10. Thoroughly wash the residue with boiling water and transfer into a Gooch crucible, prepared with a thin but compact layer of ignited asbestos.

11. Wash the residue first with boiling water and then with 15 mL of ehtyl alcohol and three successive washings with 15 mL of ether.

12. Dry the Gooch crucible content at 105 ⊃ C in air-oven for 3 hours, cool and weigh.

13. Repeat the process of drying and weighing until the difference between two consecutive weighings is less than 1 mg.

14. Incinerate the contents of Gooch crucible in an electric muffle furnace at 550 ⊃ C until all carbonaceous matter is burnt.

15. Cool the Gooch crucible in a desiccator and weigh the ash.

Calculation: Crude fibre (on dry basis) (%by weight) $= \frac{(W_1 - W_2)}{W} \times 100$

Where,

W = Weight (in gm) of the material taken for the test.

W_1 = Weight (in gm) of the Gooch crucible and contents before ashing.

W_2 = Weight of the Gooch crucible containing asbestos and ash (in gm.)

Note: Any variation in the value of crude fibre from the standard (*i.e.*, 15-30%) norm indicates contamination in the sample.

ANALYSIS OF FAT

Analysis of fat contents in a sample is done to determine its purity and level of contamination. We will examine here fat content in a butter sample.

ANALYSIS OF FAT IN BUTTER

Chemicals and Apparatus Required

1. Solvent Ether
2. Beaker
3. Glass Rod
4. Balance
5. Hot air oven

Procedure

Weigh accurately 10 g of butter in a beaker and dissolve the butter with solvent ether. Decant the ether. After complete decantation of ether, dry the residue of the beaker in hot air oven and weigh. Continue the process of drying and weighing till a constant weight is obtained.

Calculation: Percentage of fat $= \frac{(A - B)}{W} \times 100$

Where, A = weight of the beaker with sample

B = weight of the beaker after decantation of ether

W = weight of the sample taken for the test.

Note: Variation from the standard norm (*i.e.*, 1.4–3.0%) will indicate the level of contamination.

ANALYSIS OF PROTEIN

Protein is a very common ingredient of all the food materials. Hence its analysis to check the contamination becomes important. Here we will study analysis of butter and milk for protein contents.

1. Casein Content(in Butter): Protein (casein) content in butter is determined by calculation method. The total percentage of moisture, salt, lactic acid and fat is subtracted from 100. It will give the amount of protein content in butter sample in percentage.

Calculation: Percentage of Protein

= (Percentage of Moisture + Lactic acid + Fat) – 100

2. Casein Content (in Milk): Milk protein (Casein)can be separated from milk and it is possible to analyse the amount of protein by the following method:

Chemicals required	Apparatus
1. Fresh buffalo milk :200 mL	1. Beaker :2 litre capacity
2. Glacial acetic acid	2. Balance
3. NaOH Solution :0.1%	3. Hot air oven
4. Magnesium	4. Mortar
5. Distilled water	5. Muslin cloth
6. Litmus paper	
7. Rectified spirit	

Procedure

1. Dilute 200 ml fresh milk to one litre by adding distilled water in a 2 litre beaker and to this add 1 g glacial acetic acid when a white precipitate settles down.

2. Decant off the aqueous layer (do not throw it) and wash the precipitate several times with water by decantation

3. Tranfer the precipitate to a mortar and grind it with minimum amount of 0.1% sodium hydroxide solution, ncessary to neutralise the acid.

4. Test the resultant solution with litmus paper to check that amount of sodium hydroxide solution added is just sufficient to neutralise it.

5. Filter the resultant suspension through muslin cloth by pressing it hard until the liquid coming out is faintly turbid.

6. Acidify the filtrate again by adding glacial acetic acid so that the solution contains about 0.1% of it.

7. Wash the precipitate obtained by decantation with water, neutralise it with just sufficient 0.1% sodium hydroxide solution and filter through muslin cloth.

8. Repeat the process of precipitation and washing.

9. Finally drain off as much water as possible from the precipitate and make a paste of it with rectified spirit.

10. Again filter it, wash it first with alcohol and then with ether (to remove fats).

11. Dry it in air oven when casein (protein) is obtained as a white amorphous powder.

12. Weigh the yield and find out the amount of protein in gm.

CARBOHYDRATES

Carbohydrates are other nutrients commonly present in food, necessitating their analysis to check contamination. Here we will see the analysis of honey for carbohydrate contents.

ANALYSIS OF CARBOHYDRATES (OF HONEY)

Preparation of 10% honey Solution: Weigh accurately 10 g of sample and make the volume to 100 mL with distilled water in a standard flask. This is 10% honey solution.

Preparation of 1% honey solution: Pipette out 10 mL of the above prepared 10% solution into a 100 mL standard flask and make the volume to 100 mL. This will give 1% honey solution.

The above 10% and 1% honey solutions can be used for the determination of :

1. Total reducing Sugar
2. Fructose/Glucose ratio
3. Sucrose

ANALYSIS OF TOTAL REDUCING SUGARS (BEFORE INVERSION)

Apparatus required

1. Conical flask – 100 mL capacity
2. Burette
3. Pipette
4. Electric hot plate

Reagents Required

1. Soxhlet modification of Fehling's solution (Prepare by mixing equal volume of solutions *A* and *B*, immediately before use).
2. 0.5% Glucose solution.
3. Methylene blue indicator – 0.2% in water.

Procedure

1. Take the previously prepared 1% Solution (honey) in a rinsed burette.
2. Pipette out accurately 5 mL Fehling's solution A and 5 mL of solution B into a 100 mL conical flask and dilute it with 40 mL of distilled water.
3. Heat the mixture of conical flask on an electric hot plate.
4. Allow to run down the 1% honey solution from the burette into the mixture of conical flask, if possible, not more than 1 mL will be required later to complete the titration.
5. When the solution turns brick-red in colour, add 1 mL of 0.2 % methylene blue indicator solution.
6. Then add honey solution drop by drop till the end point indicated by change of colour from blue to red.
7. Note the reading of the burette.

Calculation:

$$\text{Total reducing sugar} = \frac{\text{Strength of Fehling' s solution} \times 50 \times 0.98}{\text{Titre value in the test}}$$

Note: Strength of Fehling's solution (A and B) can be determined as given below:

Fehling's solution *A*: Dissolve 69.278 g copper sulphate in distilled water and dilute to 1000 mL; Filter it through filter paper or glass wool.

Fehling's Solution B: Dissolve 346 g of Rochelle salt (sodium potassium tartrate) and 100 g sodium hydroxide in distilled water and dilute to 1000 mL. Keep the solution for a day and filter.

Standardization of Copper Sulphate: Prepare a standard 0.5% glucose solution by dissolving accurately weighed 0.5 g glucose in distilled water and dilute to 100 mL in a standard flask. Take 10 mL of Fehling's solution A and 10 mL of Fehling's solution B through a pipette into a flask and mix well. From this mixture pipette out 10 mL solution into a clean conical flask and add 40 mL distilled water. Heat this mixture on a hot plate. While heating add 9 mL of standard 0.5% glucose solution from a burette, about 1 mL less than the expected volume, which will reduce Fehling's solution completely, When the solution turns brick-red in colour, add 1 mL of

methylene blue 0.2% indicator. Then add the glucose solution drop by drop till the end point is indicated by change of colour from blue to red.

From the volume of the glucose solution used, calculate the strength of copper sulphate solution by multiplying the titre value by 0.005 (mg/mL of the standard glucose solution). This would give the quantity of the standard glucose required to reduce copper in 5 ml of copper sulphate solution.

Strength of Fehling's solution is obtained by titration against 0.5% glucose solution, 100 mL. 0.5% glucose contains 0.5 g glucose.

If x mL of 0.5% glucose solution is required for complete reduction of 10 mL of Fehling's solutions *A* and *B*.

$$\text{Strength of Fehling's solution} = \frac{0.5}{100} \times \frac{x}{10}$$

10 parts of glucose are equal to 9 parts of starch

$$\underset{\frac{C_6H_{12}O_6}{180}}{\textbf{Glucose}} \quad \times \quad \underset{\frac{(C_6H_{10}O_5)_n}{\underset{\text{mol.weight}}{162}}}{\textbf{Starch}}$$

Therefore, conversion from glucose to starch is,

$$\frac{162}{180} = \frac{9}{10} = 0.9$$

ANALYSIS OF REDUCING SUGARS (AFTER INVERSION)

Apparatus

1. Conical Flask : 100 mL capacity
2. Standard Flask : 100 mL capacity
3. Pipette
4. Burette

Chemicals Required

1. Glacial acetic Acid : 2 mL
2. Sodium carbonate
3. Red litmus Paper
4. Fehling's solution *A* and *B*

Procedure

1. Pipette out 1 mL of 10% honey solution in a 100 mL conical flask.
2. Add 2 ml of glacial acetic acid and warm to boil and keep it for two hours.
3. Neutralise it with sodium carbonate powder (check with red litmus paper to blue)
4. Make-up the solution to 100 mL in a standard flask.
5. Titrate this solution against 10 mL of Fehling's solution A and B as done earlier.

Calculation :

Reducing Sugar (After Inversion)

$$= \frac{\text{Strength of the Fehling's solution} \times 50 \times 0.98}{\text{Titre value in the test}}$$

Determination of Fructose/Glucose Ratio

Apparatus

1. Conical flask with stopper
2. Graduated pipette – 20 mL capacity

Chemicals required

1. 2N Sulphuric Acid - 44.8 mL of acid in 1000 mL distilled water.
2. N/20 Iodine Solution
3. N/10 Sodium hydroxide Solution
4. N/20 Sodium thiosulphate solution

Procedure

1. Pipette out 20 mL of 10% honey solution into a conical flask.
2. Add 40 mL of N/20 iodine (0.05 N) solution by a pipette.
3. An empty flask is also to be taken without honey sample (blank).
4. 25 mL of N/10 sodium hydroxide is added to both the stoppered flasks and kept exactly for 10 minutes in ice water bath (preferably in a dark place).
5. After 10 minutes add 5 mL of 2 N sulphuric acid to both the flasks.
6. Titrate against N/20 sodium thiosulphate solution using starch as indicator.

Calculation:

Approximate glucose

$$= \frac{0.004502 \times 100 \times \text{Strength of sodium thiosulphate} \times (B - S)}{0.2 \times 0.05}$$

where,

B = Volume of sodium thiosulphate required for empty flask (blank).

S = Volume of sodium thiosulphate required for sample

Approximate fructose % by weight

= Approximate total reducing sugar (T.R.S) (Before Inversion) – Approximate glucose

Correct glucose = (Approximate Glucose – 0.01)

$$\text{Correct Fructose} = \frac{\text{Approximate Fructose}}{0.925}$$

Correct total reducing sugar = Correct glucose + correct fructose

$$\text{Fructose/Glucose Ratio} = \frac{\text{Fructose}}{\text{Glucose}}$$

$$\text{Sucrose \% by weight} = \begin{bmatrix}\text{Reducing sugar}\\ \text{after inversion}\\ \text{\% by weight}\end{bmatrix} - \begin{bmatrix}\text{Reducing sugar}\\ \text{before inversion}\\ \text{\% by weight}\end{bmatrix}$$

ANALYSIS OF COMMERCIAL INVERT SUGAR

Presence of commercial invert sugar as adulterant in the food product can be determined by the following Method:

Fiehe's Test

Reagents required

1. Resorcinol solution : Dissolve 1 g of resublimed resorcinol in 100 mL conc. HCL.

2. Ether: Solvent ether.

Procedure

1. Take about 5 g of sugar solution in a test tube and add equal volume of solvent ether and shake gently.

2. Allow to stand until ether layer is clear.

3. Decant the ether extract into a porcelain dish.

4. Allow the extract to evaporate to dryness at room temperature.

5. Add a large drop of freshly prepared resorcinol solution.

6. A cherry red colour appearing immediately indicates the presence of commercial invert sugar.

ANALYSIS OF STARCH CONTENT

Apparatus

1. Wide mouthed conical flask of 500 mL capacity
2. Measuring flask of 500 mL capacity.
3. Burette
4. Pipette
5. Electric hot plate
6. Beaker

Reagents Required

1. Solvent Ether
2. Concentrated hydrochloric acid.
3. Sodium carbonate powder
4. Methylene blue indicator – 0.2% solution.
5. Soxhlet modification of Fehling's solution A and B. (Mix the Fehling's solutions *A* and *B* just before use)

Procedure

1. Weigh accurately 3 g sample in a beaker.
2. Dissolve the fat (if present) in solvent ether
3. Filter the ether by using filter paper.
4. Transfer the residue with filter paper to the conical distillation flask.
5. Add 25 mL concentrated HCl and 175 mL of distilled water.
6. Heat the flask on a hot plate under reflux condenser for 4 hours.
7. Cool the solution and neutralise it with sodium carbonate powder (red litmus paper turns blue).
8. Transfer the solution to a 500 mL measuring flask and make up the volume to 500 mL.
9. Filter the solution and take the clear solution for titration.
10. Take 10 mL of the Soxhlet modified Fehling's solution, add 40 mL of distilled water and titrate as given in standardisation of copper sulphate solution, and note down the volume of starch solution used in the burette.

Calculation:

$$\text{Starch \% by Weight} = \frac{\text{Strength of Fehling's solution} \times 100 \times 500 \times 0.9}{\text{Titre value} \times \text{weight of the sample taken for the test}}$$

Note: Variation from standard value (*i.e.*, more than 60 %) shows contamination in the sample.

CALCIUM

Calcium is a common constituent of food and as such its analysis for determining contamination becomes necessary. Here we will see analysis of calcium in spices.

Determination of Calcium

Reagents

1. Dilute hydrochloric Acid: 2 volumes of concentrated hydrochloric acid (relative density 1.19) diluted with 5 volumes of water.

2. Ammonium hydroxide: Ammonium hydroxide of relative density 0.90.

3. Ammonium oxalate solution: saturated

4. Standard permanganate solution: 0.1 N, standardized against sodium oxalate.

5. Dilute sulphuric acid: One volume of concentrated sulphuric acid (relative density 1.84) diluted with 4 volumes of water.

6. Bromocresol green indicator solution: 0.04% (m/v). Weigh accurately 0.1 g of bromocresol green and grind it with 14.3 mL of sodium hydroxide solution (0.01 N) in an agate mortar. Transfer the contents of the mortar quantitatively to a 250 ml graduated flask and make up the volume with water.

7. Acetic acid: one volume of glacial acetic acid diluted with 2 volumes of water.

Procedure

1. Weigh about 2 to 4 g of the material and obtain total ash as given earlier (in analysis of total ash).

2. Digest the ash in the dish with dilute hydrochloric acid.

3. Evaporate to dryness. Digest the dry residue again with dilute hydrochloric acid, and again evaporate to dryness on a water bath.

4. Treat the residue with 5 to 10 mL concentrated hydrochloric acid, add about 50 mL of water, allow to stand on water-bath for a few minutes and filter in a 250 mL beaker.

5. Wash the insoluble residue with hot water and collect the washings in the same beaker.

6. Add to the solution in the beaker 0.5 mL of bromocresol green indicator and then the ammonium hydroxide till the colour of the solution is distinctly blue. Adjust the pH of the solution at 4.4 to 4.6 by adding acetic acid drop by drop until the colour changes to distinct green.

7. Filter it and wash the filter paper with hot water. Collect the washings in the same beaker and bring the solution to boil.

8. While still hot, add saturated ammonium oxalate solution drop-wise as long as any precipitate forms and then add excess. Heat to boiling.

9. Allow to stand for 3 hours or longer.

10. Decant the clear solution through an ashless filter paper.

11. Pour 15 to 20 mL of hot water on the precipitate and again decant the clear solution. Dissolve any precipitate remaining on the filter paper by washing with hot dilute hydrochloric acid into the original beaker. Wash the filter paper thoroughly with hot water.

12. Then reprecipitate, while boiling hot, by adding ammonium hydroxide and a little of the ammonium oxalate solution.

13. Allow to stand for 3 hours or longer as before, filter through the same filter paper, and wash with hot water until it is chloride free.

14. Perforate the apex of the filter cone. Wash precipitate into the beaker used for precipitation. Then wash the filter paper with hot dilute sulphuric acid and titrate at a temperature not less than 70⊃C with standard potassium permanganate solution.

Calculation : Calcium (as CaO), percent by mass $= \frac{2.8\,VN}{M}$

where,

V = volume in ml of the standard potassium permanganate solution used for titration.

N = Normality of the standard potassium permanganate solution.

M = Mass in g of the material taken for the test.

SODIUM

Sodium is an important element available in food. We will see its presence in chloride form in a sample of spice.

Determination of Sodium Chloride

Reagents

1. Dilute nitric acid : one volume of concentrated nitric acid (relative density 1.42) diluted with 4 volumes of water and freed from lower oxides of nitrogen by boiling until colourless.

2. Standard silver nitrate solution : 0.1N

3. Ferric indicator solution : saturated solution of ferric ammonium sulphate [$FeNH_4(SO_4)_2 \cdot 12H_2O$]

4. Potassium thiocyanate solution – 0.1 N

Procedure

1. Weigh accurately about 5.0 g of the material sample in a dish preferably of platinum and obtain the total ash as described earlier.

2. Dissolve the ash in hot water. Filter and wash the dish and residue thoroughly with hot water till it is free from chlorides.

3. Collect the filtrate and washings in an Erlenmeyer flask.

4. Add a known volume of the standard silver nitrate solution in slight excess, 5 mL of the ferric indicator solution and a few mL of nitric acid.

5. Titrate the excess silver nitrate with the standard potassium thiocyanate solution until permanent light brown colour appears.

Calculation: Sodium Chloride, percent by mass $= \frac{5.85(V_1N_1 - V_2N_2)}{M}$

where,

V_1 = Volume in mL of the standard silver nitrate solution used.

N_1 = Normality of the standard silver nitrate solution.

V_2 = Volume in mL of the standard potassium thiocyanate solution used.

N_2 = Normality of the standard potassium thiocyanate solution

M = Mass in g of the material taken for the test.

Note : Variation from standard value (i.e. 5.0-10.0%) indicates the presence of adulteration in sample.

Standard Values of Some Food Materials

S.No.		Oils	Spices	Butter/Ghee	Honey
1.	Moisture (% by weight)	0.1–1.0	10–12.5	less than 0.2%	20–25
2.	Ash (Total) (% by weight)	–	7.0–9.0	–	0.5
3.	Ash (acid insoluble) (% by weight)	–	1.0–1.5	–	–
4.	Fat	–	–	1.4–3.0	–
5.	Crude fibre (% by weight)	–	15.0–30.0	–	–
6.	Carbohydrates (a) Starch (% by weight) (b) Total reducing sugars (% by weight) (c) Fructose/glucose ratio	– – – –	60.0 – –	– – –	– 60.0–65 0.9–1.0
7.	Sodium chloride	–	5.0–10.0	–	–

ADULTERANTS

A substance in its pure form is said to be free from adulteration. That is to say, when something else gets mixed up with the pure substance, it can be said to be adulterated with the material mixed with it.

Adulteration, generally, is deliberate mixing of cheap material of low value with a substance of higher value with a view to increase its volume, weight or quantity. Materials used for adulteration are very much similar in look, colour etc. to the real substance to make the detection difficult by visual, odour or ordinary examination. It is mostly practised by unscrupulous people to make undue gain and profit. Therefore the need for analysis of food to check adulteration arises.

Common examples of adulteration are:

1. Kerosene mixed with petrol to increase the volume of higher valued petrol.
2. Sugar added to honey which is much more valuable.
3. Vegetable ghee in pure ghee, and
4. Crude fibres or starch etc. in spices, etc.

CONTAMINANTS

As opposed to adulteration, the contaminants can be said to be impurities getting mixed up in a pure substance due to wrong handling or processing etc. Thus, whereas adulteration is done deliberately, contamination is often accidental.

The examples of contamination can be:

1. Growth of fungi in food materials, and
2. Earth and stone particles in food grains and pulses etc.

MICROSCOPIC EXAMINATION OF FOOD

The microbiological safety of food is very important to the food industry. All food-stuffs including dairy products and meals, meats, vegetables, herbs and spices have potential micro-biological hazards associated with them. There are many laboratories which use conventional common techniques such as using the selective broths and agar plates to identify the bacteria and moulds, which are responsible for food poisoning or spoilage. These techniques are slow, require a great care and are usually performed by an expert microbiologist.

Now-a-days, scientists have discovered many advanced tests and analyses for detection of microbes in a wide range of foods. These microscopic examinations are performed by adding enriched food samples to a microtitre plate. The plate is washed and a highly specific monoclonal antibody conjugate is added to it, which binds to microbe antigens forming an immune complex. This plate is washed again. The second washing removes any unbound conjugate. The presence/absence of microbe is determined by the addition of a colourless substrate, which produces a coloured reaction in the presence of that particular micro-organism. In this manner the result can be determined on a microplate reader at 450 nm and it can be read visually.

COMMON MICRO-ORGANISMS OF FOOD STUFFS

There are some common micro-organisms found in food products, such as:

1. Salmonella 2. Listeria 3. Zearalenone 4. Fumonisins
5. Ochratoxins 6. Aflatoxins 7. Mycotoxins 8. Deoxynivalenol
9. Trichothecenes

1. Salmonella: Salmonella are pathogenic bacteria, which are found in a wide range of foods. Salmonella are enteric organisms, occurring naturally in the gut of poultry and animals, therefore the live stock show no signs of illness or disease.

Disease

In human being Salmonella causes severe sickness and diarrhoea. In children, old people and immunosuppressed individuals the effect of Salmonella food poisoning (Salmonellosis) can be more severe leading to septicemia, dehydration and even death.

2. Listeria: Listeria are pathogenic bacteria, which are found mainly in the environment, particularly in water and soil. Listeria are often found in dairy products such as cheese and yoghurt but can also be found in meat, pate, vegetables and salad. Listeria are able to grow at low temperature of 4⊃C and therefore can survive even in food which has been stored in a refrigerator.

Disease

Listeria generally cause mild flu like symptoms in healthy adults but can have more serious effects on the foetus of pregnant women causing neonatal septicemia or abortion. Women are, therefore, advised during pregnancy to avoid eating soft cheese and pate.

3. Zearalenone: Zearalenone is an oestrogenic mycotoxin produced by a number of species of fusarium. Zearalenone readily colonise cereals such as barley, wheat, rice and maize during cool and wet growing seasons. Zearalenone has also been found in breakfast cereals, breads and animal feed products.

Disease

Zearalenone acts by mimicking the effect of the female hormone oestrogen, causing adverse effects on the mammalian reproductive system resulting in infertility.

4. Fumonisins: Fumonisins are a group of mycotoxins produced by various fusarium species, the most common being F. moniliforme and F. prdiferatum. There are three types of fumonisins commonly found in food: B_1, B_2, and B_3. The most important toxin in the group is Fumonisins B_1, the concentration of which usually exceeds that of B_2 and B_3 by a factor of three.

Occurrence

Fumonisins are commonly found in maize, maize based products and rice. Examples of maize based products include popcorn, cornflour, cornflakes and baby food. Fumonisins occur in different climates and can be found at high levels in commodities from USA, Canada, Africa and Europe.

Disease

Fumonisins are known to be hepatoxic, nephrotoxic and carcinogenic to rats and mice. Fumonisins in feed can cause necrosis of the brain in horses, which is often fatal, and swelling of the lungs and thorax in pigs.

5. Ochratoxins: Ochratoxins are produced by various moulds of the genera Aspergillus and Penicillium. Ochratoxin A is the most important and most commonly occuring Ochratoxin, although ochratoxin B has also been found to occur naturally in food. Ochratoxin A occurs primarily when foods with a high water content are not properly dried, creating the ideal environment for the mould to reproduce. Ochratoxin A can be commonly found in cereals, coffee, cocoa, wine, spices, beer, barley. Due to Ochratoxin A occurring as a result of a common storage fungus, contaminated commodities are usually found in temperate areas such as Eastern, Western and Northern Europe, Canada and South America. Ochratoxin A is also commonly found in the viscera of animals that have consumed contaminated grains.

Disease

When present in animals, both acute and chronic lesions are observed on the organs, primarily the kidneys. Ochratoxin A also has teratogenic and foetotoxic effects in animals and some studies have also suggested that it may have immunotoxic and possibly neurotoxic effects.

6. Aflatoxins: Aflatoxins are produced by the food spoilage fungi Aspergillus flavus and Aspergillus parasiticus. There are approximately 20 related fungal metabolites, although only aflatoxins B_1, B_2, G_1, G_2, and M_1 are normally found in foods. Aflatoxins can be found in a variety of foods and agricultural commodities including cereals, nuts, herbs, dairy products, spices, and dried fruits. They are generally formed in tropical and sub-tropical climates, where growing temperatures are high and storage conditions can be humid. Aflatoxins are extremely stable and may remain in food stuff even after processing (such as roasting) and the removal of visible mould.

Disease

Aflatoxins have been found to have mutagenic, immuno suppressive and carcinogenic effects in both human and animals.

7. Mycotoxins: Mycotoxins are toxic metabolites produced by different species of the readily colonized crops in the field or after harvest, during

storage. Mould growth can depend on a number of factors for example temperature, humidity, weather conditions during growth, harvest and insect activity. Mycotoxins have been found to contaminate a number of commodites including cereals, dried fruits, nuts, dairy products, animal feed, cocoa, spices, wine, beer and coffee. European and world wide legislation is setting maximum limits for the range of mycotoxins in a variety of foods and feed commodities.

Disease

Mycotoxins can have both chronic and acute effects on human and animal health. They can be carcinogenic, immunosuppressive, embryotoxic, and mutagenic.

8. Deoxynivalenol: Deoxynivalenol, also known as DON or vomitoxin, is part of a group of related compounds known as Trichothecenes. Trichothecenes are formed by a number of species of the fusarium genera, including F. graminearum and F. culmorum. The main commodities affected by DON contamination are cereals, including grains such as wheat, buckwheat, oats, rice, maize and barley. Due to its stability, DON has also been detected in a range of processed cereal products including breakfast cereals, beer, infant food, and bread. The formtion of DON in growing crops is dependent on climate and varies between geographical regions and parts of the year.

Disease

Digestion of DON contaminated commodities is known to induce vomiting, particularly in pigs, feed refusal, weight loss and diarrhoea. Studies also suggest that DON may also have effects on the immune system.

9. Trichothecenes: Trichothecenes are the largest group of mycotoxins and are produced by fusarium moulds. There are over 40 different trichothecenes including deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), fusarenon –X (FX), T–2 and HT–2. 3–Acetyl DON and 15–Acetyl DON, derivatives of DON also exist in nature. Trichothecenes occur in cereals including wheat, maize, barley, oats and rice and they are present in many different climates. In some parts of the world, such as the USA and Canada, DON and NIV are endemic.

Deoxynivalenol is one of the most common and prevalent among the trichothecenes.

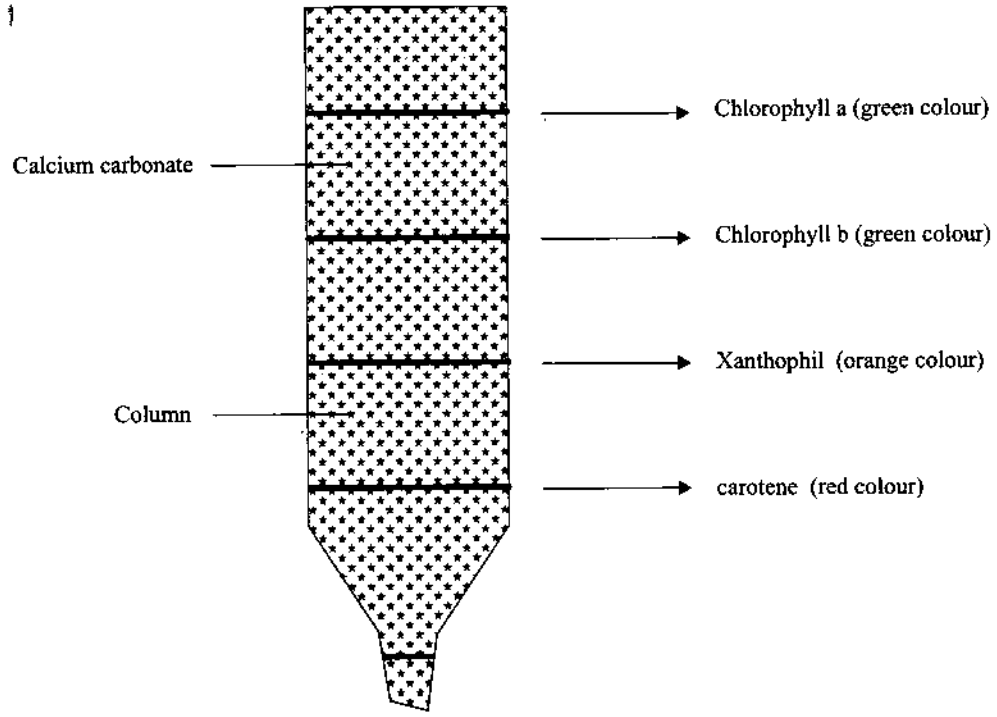
Disease

DON is also called vomitoxin due to the fact that it causes sickness in human and animals (for example swine will refuse feed containing DON). DON can also have adverse effects on the immune response and is considered to have the potential to predispose human and animals to other diseases. Trichothecenes received their notoriety in Russia following world War II due to the discovery of T–2 toxin that caused internal bleeding among human who ingested over wintered cereals in bread. T–2 and diacetoxyscirpenol (DAS) have also been found to be highly toxic if taken orally or through penetration of the skin, they can also cause mouth lesions and acute poisoning.

CHROMATOGRAPHY

Chromatography is a modern separation technique which can resolve a multicomponent mixture into its individual fractions.

The term chromatography means—'Chromatos' = colour and 'graphos' = writing, it was discovered by a Russian botanist, Mikhail Semyonovich Tswett in 1901. In this technique, separation of a mixture of substances is brought about by differential movement of individual components through a porous medium in the presence of a solvent. For the first time Tswett separated green plant-extract in to its pigments by using a powdered packed-calcium carbonate column and then washed with light petroleum, he found a series of coloured horizontal bands called chromatogram on the column as shown below.



Separation of Plant-Extract by column chromatography.

Since the discovery of this technique, it has undergone many changes and is now a very sophisticated technique.

Some Definitions of Chromatography

1. Tswett has defined chromatography 'As a process used in separating substances by filtering their solution through a column of a finely powdered adsorbent and then developing the column with a solvent.'

2. Martin has defined that "chromatography is an analytical technique for separation of a mixture of solutes, brought about by the dynamic partition or distribution of dissolved materials between two immiscible phases."

3. According to Strain "Chromatography is the name given to the technique for the resolving of solutes, in which separation is made by differential migration in a porous medium and the migration is caused by flow of solvent".

4. Chromatography is a separation process applicable to molecular mixtures, in which the distribution of mixture occurs between two dimensional phases which are brought into contact in a differential counter current manner".

Principle : Chromatography is based upon the principle of selective adsorption of various components of a mixture between two phase, **one fixed phase** (stationary phase), and the other **moving phase** (mobile phase). The mixture to be separated is taken in the moving phase and passed over the fixed phase. Different components are adsorbed to different extents by the fixed phase. This results in separation of the mixture.

Types of Chromatographic Methods

In all the chromatographic techniques, difference in affinity involves the two processes (a) adsorption (b) partition.

(a) Adsorption chromatography : In adsorption chromatographic technique a solid as the stationary (fixed) phase and a liquid or gas as a moving phase (mobile) is involved. In column chromatography, a solid adsorbent is taken in a column, whereas in thin layer chromatography (T.L.C.) the adsorbent is supported on a glass plate.

(b) Partition chromatography : When the chromatographic technique involves a liquid supported on an inert solid as stationary phase and a liquid or gas as moving phase, it is a case of partition chromatography. Paper chromatography is an example of partition chromatography. Here paper acts as an inert support.

Some advanced chromatographic methods are as :

1. Ion-Exchange chromatography : This includes

(a) Cation-Exchange chromatography

(b) Anion-Exchange chromatography

(c) Liquid-Exchanger

(d) Inorganic-Exchanger

2. Exclusion chromatography : It includes

(a) Gel-Chromatography

(b) Ion-exclusion chromatography

(c) Molecular sieve

3. Electrochromatography : It consists of

(a) Zone electrophoresis

(b) Boundary layer method

(c) Curtain chromatography

(d) Capillary Electrophoresis

High performance liquid chromatography (HPLC) does not classify in the above categories since it is highly advanced instrumental technique.

Various chromatographic methods consist of a few techniques in common. There is a stationary support in the form of a column packed with inert material, a phase moving down the column known as mobile phase and the phase which adheres to the stationary support called the stationary phase. Separation is possible on account of a differential migration front, which developed by exploiting differences in adsorption, partition coefficient, molecular size, ionic mobility and exchange potential.

Chromatography

Adsorption chromatography			Partition chromatography		
Stationary Phase	*Mobile Phase*	*Example*	*Stationary Phase*	*Mobile Phase*	*Example*
Solid	Gas	Gas-Solid chromatography (GSC)	Liquid	Gas	Gas-liquid chromatography (GLC)
Solid	Liquid	(a) Column chromatography and (b) Thin layer chromatography (TLC)	Liquid	Liquid	(a) Paper chromatography (PC) (b) Column Chromatography (c) Thin layer chromatography (TLC) (d) Reversed Phase partition chromatography (RPPC) (e) Gel-Permeation (f) Ion-Exchange chromatography

PAPER CHROMATOGRAPHY

1. Introduction

The paper chromatography separation techniques discovered by the Cambridge school of workers, **A.J.P. Martin, R. Consden, A.H. Gordon** and **R.L.M. Syngle.**

2. Principle

This technique is a type of partition chromatography in which the substances are distributed between two liquids, *i.e.*, one is the stationary liquid (usually water) which is held in the fibres of the paper and called the *stationary phase;* the other is the moving liquid or developing solvent and called the moving phase. The components of the mixture to be separated migrate at different rates and appear as spots at different points on the paper.

Formerly paper chromatography was used to separate mixture of organic substances such as dyes and amino acids only. But now this method has been used to separate cations and anions of inorganic substances as well.

In this technique, a drop of the test solution is applied as a small spot on a filter paper and the spot is dried. The paper is kept in a close chamber and the edge of the filter paper is dipped into a solvent called *developing solvent.* As soon as the filter paper gets the liquid through its capillary action and when it reaches the spot of the test solution the substances are moved by solvent system at various speeds. When the solvent has moved these cations to a suitable height (15-18 cm) the paper is dried and the various spots are visualised by suitable reagents called *visualising* reagents. The movement of substances relative to the solvent is expressed in terms of R_F values, *i.e.*, migration parameters.

Migration parameters

The position of migrated spots on the chromatograms are indicated by different terms such as R_F , R_x , R_M , and R_C.

R_F : The R_F is related to the migration of the solute front relative to the solvent front as :

$$R_F = \frac{\text{Distance travelled by the solute from the origin line}}{\text{Distance travelled by the solvent from the origin line}}$$

R is a function of the partition coefficient. It is a constant for a given substance, provided the conditions of chromatographic system are kept constant with respect to temperature, type of paper, duration and direction of development, nature and the shape and the size of the wick used (*i.e.*, radial chromatography), the amount of liquid in the reservoir, humidity, etc.

The R_F defines the movement of the substance relative to the solvent front in a given chromatographic system.

The R_F value of a substance depends upon a number of factors which are as follows :

(i) The solvent ,

(ii) The medium used for separation, *i.e.*, the quality of paper in case of paper chromatography,

(iii) The nature of the solute mixture,

(iv) The temperature,

(v) The size of the vessel in which the separation is carried out.

R_X : In some times the solvent front runs off till the end of filter paper, the movement of a substance in such cases is expressed as R_X rather than R_F

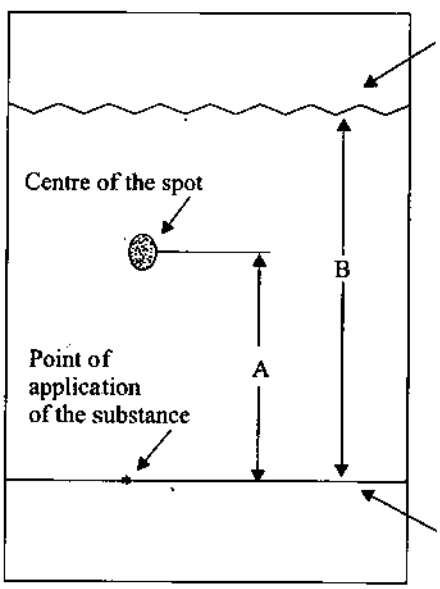
$$R_X = \frac{\text{Distance travelled by the substance from the origin line}}{\text{Distance travelled by the standard substance x from the origin line}}$$

R_F and R_X have been illustrated below.

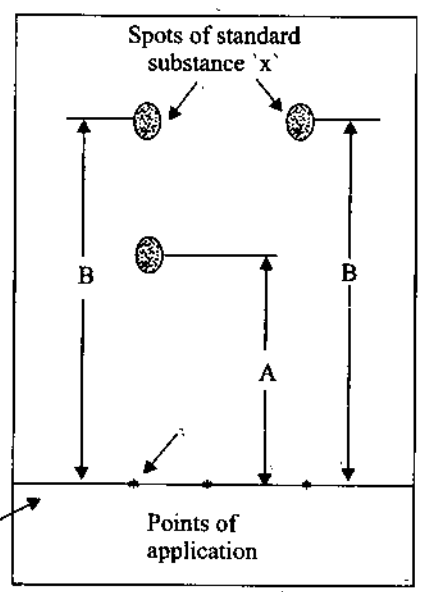
R_M : According to **Bate, Smith** and **Westall**, R_M is defined as follows :

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

The term R_M is additive and is composed of the partial R_M values of the individual functional groups or other groupings of atoms in the molecules.



Diagramatic representation of R_F

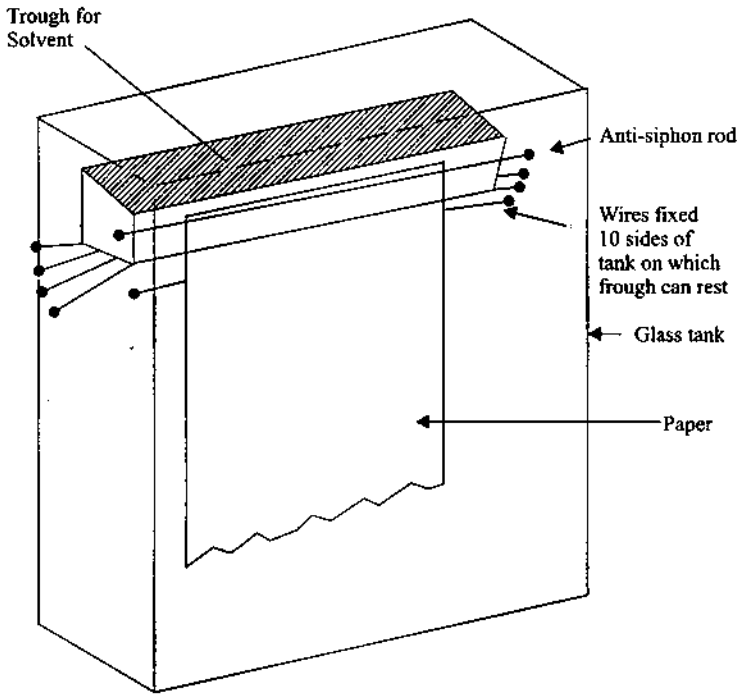


Diagramatic representation of R_X

Types of Paper Chromatography

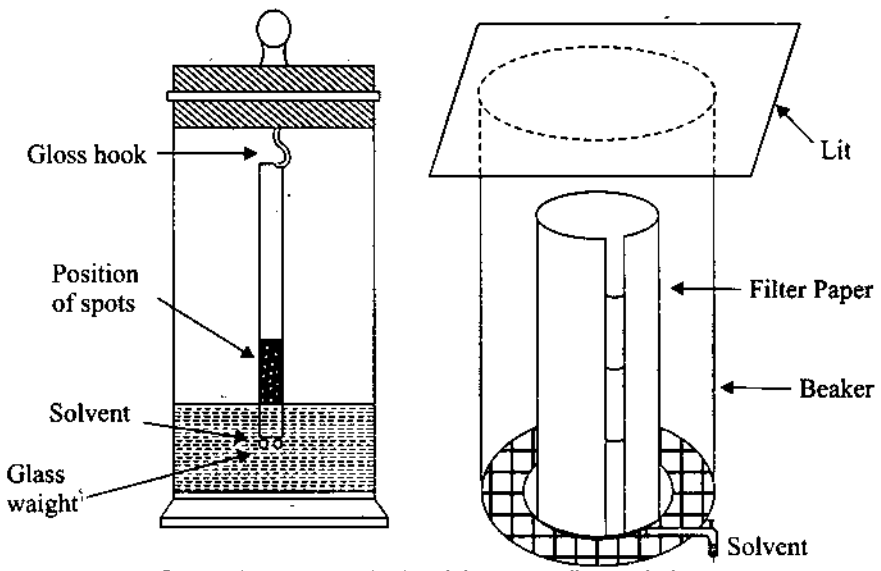
1. Descending Chromatography : When the development of the paper is done by allowing the solvent to travel down the paper, it is known as *descending technique*. The apparatus consists of a well-sealed glass tank with a trough for the mobile phase in the upper portion. The paper with the sample spotted is inserted with the upper end in the trough containing the mobile phase, the jar itself equilibrated with the mobile phase before the elution.

The advantage of descending technique is that the development can be continued indefinitely even though the solvent runs off at the other end of the paper.



Apparatus descending chromatography.

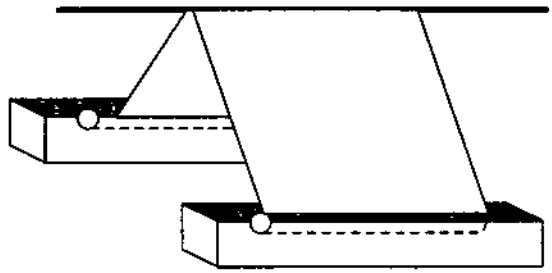
2. Ascending chromatography. When the development of the paper is done by allowing the solvent to travel up the paper, it is known as ascending technique.



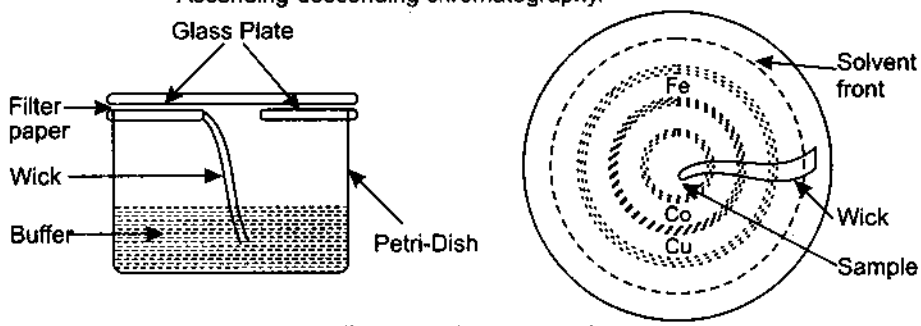
Paper chromatography involving ascending technique.

In ascending chromatography, the mobile phase is kept in a container at the bottom of the chamber. The samples are aplied a few centimeters from the bottom edge of the paper suspended from a hook. Alternatively the paper may be rolled into a cylinder, held together by staples, strings or plastic clips.

Both ascending and descending techniques have been used for separation of organic and inorganic substances. But the descending technique is prefered if the R_F values of various constituents are almost same.



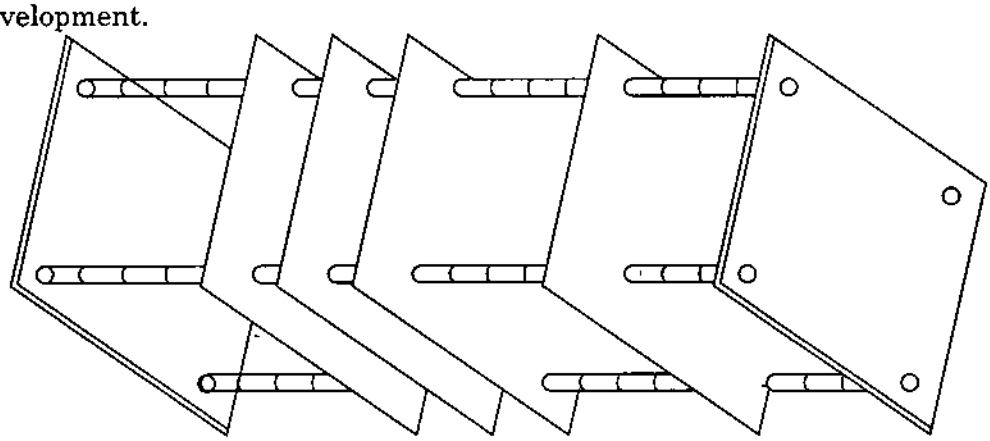
Ascending-descending chromatography.



Radial paper chromotography.

3. Ascending-Descending Chromatography : It is the mixture of the above two techniques. In this technique, the upper part of the ascending chromatography can be folded over a glass rod allowing the ascending development to change over into the descending after crossing the glass rod.

4. Radial Paper Chromatography : This is also known as circular paper chromatography. In this technique a circular filter paper is employed. The materials to be analysed are placed at its center. After drying the spot the paper is fixed horizontally on the petri-dish possessing the solvent so that the wick of the paper dips into the solvent. Cover the paper by means of petri-dish cover. The solvent rises through the wick. When solvent front has moved through a sufficient large distance, the components get separated in the form of concentric circular zones. This chromatography provides use of radial development.



Rack arrangement for two-dimensional chromatography.

5. Two dimensional chromatography : In this technique a square or rectangular paper is used. The sample is applied to one of the corners. The second development is performed at right angle to the direction of the first run. This type of chromatography can be carried out with identical solvent systems in both the directions or by two solvent systems. Rack arrangement for the two dimensional chromatography is shown below.

Experimental Details

1. Choice of the Chromatographic Technique : The first job is to select the mode of paper chromatographic technique, *i.e.*, ascending, descending, ascending- descending, radial or two-dimensional technique. The choice of technique depends upon the nature of the substances to be separated.

2. Choice of the Filter Paper : The filter paper plays an important role in the success of paper chromatography. The choice of paper is dependent on the factors which are as follows :

(i) Whether the paper is used for quantitative or qualitative analysis;

(ii) Whether it is used for analytical or preparative chromatography;

(iii) Whether the solutes used are hydrophilic or lipophilic neutral or charged species.

Different types of *Whatmann* chromatography papers are available. The choice of a particular *Whatmann* chromatographic paper depends upon the type of separation. Generally, coarser and faster papers, *i.e.*, Whatmann 31 ET, are used when substances to be separated are sufficiently wide-apart R_F values. Slow papers are rarely used. However, they facilitate a better resolution of substances with close R_F values. The slow papers available are *Whatmann* 20 *Schleicher* and *Schull* 2045. Heavy papers like *Whatmann* 3 mm are used for preparative purpose. With *Schleicher* and *Schull* 2071, a single sheet has been used for the separation of gram quantities of solutes.

Different types of *Whatmann* Chromatography papers are described below :

Types of Whatmann Chromatographic Papers

Papers	*Rate of Flow*		
	Fast	*Medium*	*Slow*
Thin papers	No. 4 No. 54 No. 540	No. 7 No. 1	No. 2 No. 20
Thick Papers	No. 31 No. 17	No. 3 No. 3MM	

Whatmann filter papers commonly used for chromatographic purposes consist of 99% of α-cellulose. The rest is mineral content.

For the efficient and fast separation of polar substances, the exchange capacity of the paper is increased by increasing the carboxyl content (1.4%) using partial oxidation.

The capillary action of the paper can be improved by partial hydrolysis for 24 hours in 7% hydrochloric acid followed by washing with water and ethanol.

It is possible to increase the rigidity of the paper by acetylation, esterification, and other chemical methods.

3. Proper developing solvent : The choice of this depends upon the fact that R_F values should be different for different constituents present in a

mixture. Generally a solvent or solvent mixture which gives a R_F of 0.2–0.8 for the sample should be selected for the better separation.

The various solvents which are generally used in paper chromatography are listed below, according to their polarity. If a pure solvent is not sufficient for the separation, a mixture in different proportions can be prepared and used for efficient resolution of substances.

Solvent for paper chromatography in Order of Increasing Polarity

Solvent	t_{20}	t_{25}
n-hexane	1.89	
Cyclohexane	2.02	
Carbon tetrachloride	2.24	
Benzene	2.29	
Toluene	2.44	
Trichloroethylene	3.40	
Diethyl ether	4.34	
Chloroform	4.91	
Ethyl acetate		6.02
n-butanol	17.80	17.10
n-propanol		20.10
Acetone		20.10
Ethanol		24.30
Methanol		32.60
Water	80.40	78.50

4. Preparation of samples : The sample volume of 10–20 μ having as many μg of the substance is the ideal quantity to be spotted on paper.

5. Spotting : For ascending technique, a strip of Whatmann filter paper of suitable size (25 cm × 7 cm) is generally used. A horizontal line is drawn on the filter paper by a lead pencil. This line is known as *origin line*. On the origin line, cross marks (x) are made with a pencil in such a way that each cross (x) is at least 2 cm away from other as shown below. With the help of a graduated micro pipette the test solutions are applied on cross (x) marks and the spots are dried by a stream of hot or cold air.

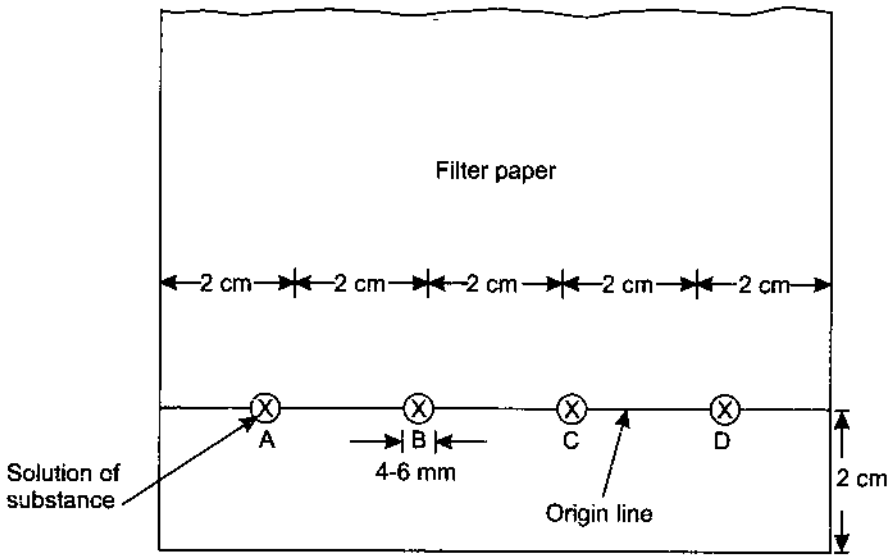
6. Drying the Chromatograms : The wet chromatograms after development are dried in special drying chambers which are being heated electrically with temperature controls.

7. Visualisation : Visualisation of the spots can be processed by two methods :

(i) Either by chemical means or

(ii) By using pysical methods.

(i) Chemical Method : Chemical treatment can develop the colour by colourless solvents on the paper. The reagents used for visualising the spots are known as *chromogenic reagents* or *visualising* agents.



The reagents are applied in one of two manners either by the pressure spraying or by dipping the chromatogram. Reagents in organic solvents are more suitable for spraying than aqueous solutions.

(ii) Physical Method : Some colourless spots, when placed under UV lamp, produce flourescence and shows their presence. If the substance is coloured, the spots can be observed by reflected or transmitted light.

8. Calculation of R_F values : The distance of chromatographed spots is measured from its centre to the origin line. The distance of solvent front from the origin line gives the R_F values.

COLUMN AND THIN-LAYER LIQUID CHROMATOGRAPHY

INTRODUCTION

The term 'liquid chromatography' consists of a variety of separation techniques, such as liquid-solid, liquid-liquid, ion exchange and size-exclusion chromatography, all involving a *liquid mobile phase.* Classical liquid column chromatography is characterised by use of relatively wide-diameter glass columns, packed with a finely divided stationary phase with the mobile phase percolating through the column under gravity. Although many separations have been achieved, these are generally slow and examination of the recovered fractions (*e.g.* by chemical or spectroscopic techniques) can be tedious. Since about 1969 the development of modern high-performance liquid chromatography (HPLC) has enabled liquid chromatography to match the great success achieved by gas chromatography in providing the following features :

(a) high resolving power;

(b) speed of separation;

(c) continuous monitoring of the column effluent;

(d) accurate quantitative measurement;

(e) repetitive and reproducible analysis using the same column; and

(f) automation of the analytical procedure and data handling.

High-performance liquid chromatography is in some respects more versatile than gas chromatography because (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider.

TYPES OF LIQUID CHROMATOGRAPHY

There are four main types of liquid chromatography which require discussion.

1. Liquid-solid chromatography (LSC) : This process, often termed *adsorption chromatography,* is based on interactions between the solute and fixed active sites on a finely divided solid adsorbent used as the stationary phase. The adsorbent, which may be packed in a column or spread on a plate, is generally a high surface area, active solid such as alumina, charcoal or silica gel. Highly active adsorbents may give rise to irreversible solute adsorption; silica gel, which is slightly acidic, may strongly retain basic compounds, whilst alumina (non-acid washed) is basic and should not be used for the chromatography of base-sensitive compounds. Adsorbents of particle size, *e.g.* 20–40 μm for TLC and less than 5 μm for HPLC, may be purchased commercially.

The role of the solvent in LSC is clearly vital since mobile-phase (solvent) molecules compete with solute molecules for polar adsorption sites. The stronger the interaction between the mobile phase and the stationary phase, the weaker will be solute adsorption, and vice versa. The classification of solvents according to their strength of adsorption is known as an eluotropic series, which may be used as a guide to find the optimum solvent strength for a particular separation. Solvent purity is very important in LSC since water and other polar impurities may significantly affect column performance, and the presence of UV-active impurities is undesirable when using UV-type detectors.

The compounds best separated by LSC are those which are soluble in *organic solvents and* are *non-ionic. Water soluble non-ionic* compounds are better separated using either reverse-phase or bonded-phase chromatography.

2. Liquid-liquid (partition) chromatography (LLC). This type of chromatography is similar in principle to solvent extraction and based upon the distribution of solute molecules between two immiscible liquid phases according to their relative solubilities. The separating medium consists of a finely divided inert support (*e.g.* silica gel, kieselguhr, etc.) holding a fixed (stationary) liquid phase, and separation is achieved by passing a mobile phase over the stationary phase. The stationary phase may be in the form of a packed column, a thin layer on glass, or a paper strip.

It is convenient to divide LLC into two categories, based on the relative polarities of the stationary and mobile phases. The term *'normal LLC'* is used when the stationary phase is *polar* and the mobile phase is ***non-polar***. In this case the solute elution order is based on the principle that non-polar solutes prefer the mobile phase and elute first, while polar solutes prefer the stationary phase and elute later. In **reverse-phase chromatography** (RPC) the stationary phase is non-polar and the mobile phase is polar and the solute elution order is reverse as observed in normal LLC, i.e. with polar compounds eluting first and non-polar ones later. This is a popular mode of operation due to its versatility and scope. The almost universal application of RPC arising from the fact that all organic molecules have hydrophobic regions in their structure and are therefore capable of interacting with the non-polar stationary phase. As the mobile phase in RPC is polar, and contains water, the method is particularly suited to the separation of polar substances which are either insoluble in organic solvents or bind too strongly to solid adsorbents

(LSC) for successful elution. Some typical stationary and mobile phases which are used in normal and reverse phase chromatography are given below :

Typical stationary and mobile phases for ***normal*** and ***reverse phase chromatography***

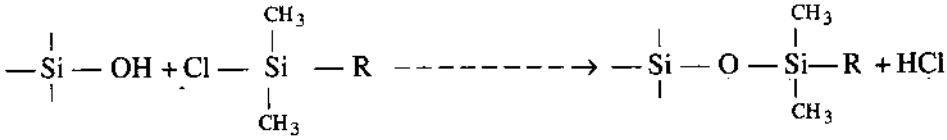
Stationary phases	Mobile phases
Normal	
β, β′-Oxydipropionitrile Carbowax (400, 600, 750, etc.) Glycols (ethylene, diethylene) Cyanoethylsilicone	Saturated hydrocarbons, *e.g.* hexane, heptane; aromatic solvents, *e.g.* benzene, xylene; saturated hydrocarbons mixed with up to 10 per cent dioxan, methanol, ethanol, chloroform, methylene chloride (dichloromethane)
Reverse-phase	
Squalane Zipax-HCP Cyanoethylsilicone	Water and alcohol-water mixtures; acetonitrile and acetonitrile-water mixtures

Although the stationary and mobile phases in LLC are chosen to have as little solubility in one another as possible, even slight solubility of the stationary phase in the mobile phase may result in the slow removal of the former as the mobile phase flows over the column support. For this reason the mobile phase must be pre-saturated with stationary phase before entering the column. This can be done by using a pre-column before the chromatographic column. The pre-column should contain a large-particle packing (*e.g.* 30–60 mesh silica gel) coated with a high percentage (30–40 per cent) of the stationary phase to be used in the chromatographic column. As the mobile phase passes through the pre-column it becomes saturated with stationary phase before entering the chromatographic column.

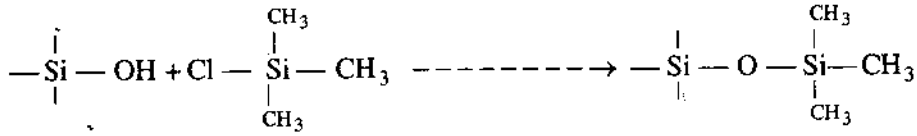
The *ion-pair chromatography* (IPC) is essentially a partition-type process analogous to the ion-association systems used in solvent extraction. In this process the species of interest associates with a counter ion of opposite charge, this opposite charged ions selected to confer solubility in an organic solvent on the resulting ion pair. The technique can be used for a wide variety of ionisable compounds but particularly for those yielding large aprotic ions, *e.g.* quaternary ammonium compounds, and for compounds such as amino acids which are difficult to extract in the uncharged form. The stationary phase consists of an aqueous medium containing a high concentration of a counter ion and at a suitable pH, typical support materials being cellulose, diatomaceous earth and silica gel. The mobile phase is generally an organic medium having low to moderate solvating power. A useful advantage of the ion-pair technique is the possibility of selecting counter ions which have a high response to specific detectors, *e.g.* counter ions of high molar absorptivity like bromothymol blue, or highly fluorescent anions such as anthracene sulphonate.

3. Bonded-phase chromatography (BPC). To overcome some of the problems associated with conventional LLC, such as loss of stationary phase from the support material, the stationary phase may be chemically bonded to the support material. This form of liquid chromatography, in which both *monomeric* and *polymeric phases* have been bonded to a support materials, is termed *'bonded-phase chromatography'*.

Silylation reactions have been used to prepare bonded phases. The silanol groups (—Si — OH) at the surface of silica gel are reacted with substituted chlorosilanes. A typical example is the reaction of silica with a dimethylchlorosilane which produces a monomeric bonded phase, as each molecule of the silylating agent can react with only one silanol group :



The use of di-or tri-chlorosilanes in the presence of moisture can result in a polymeric layer being formed at the silica surface, *i.e.* a polymeric bonded phase. Monomeric bonded phases are preferred as they are easier to manufacture than the polymeric type. The nature of the main chromatographic interaction can be varied by changing the properties of the functional group R; in analytical HPLC the most important bonded phase is the non-polar C-18 type in which the modifying group R is an octadecyl hydrocarbon chain. Unreacted silanol groups are capable of adsorbing polar molecules and will affect the chromatographic properties of the bonded phase, it sometimes producing undesirable effects such as tailing in RPC. These effects can be minimised by the process of 'end-capping' where these silanol groups are rendered inactive by reaction with trimethylchlorosilane.



An important characteristic of these siloxane phases is their stability under the conditions used in most chromatographic separations; the siloxane bonds are attacked only in very acidic (pH < 2) or basic (pH > 9) conditions. A large number of commercial bonded-phase packings are available in particle sizes which are suitable for HPLC.

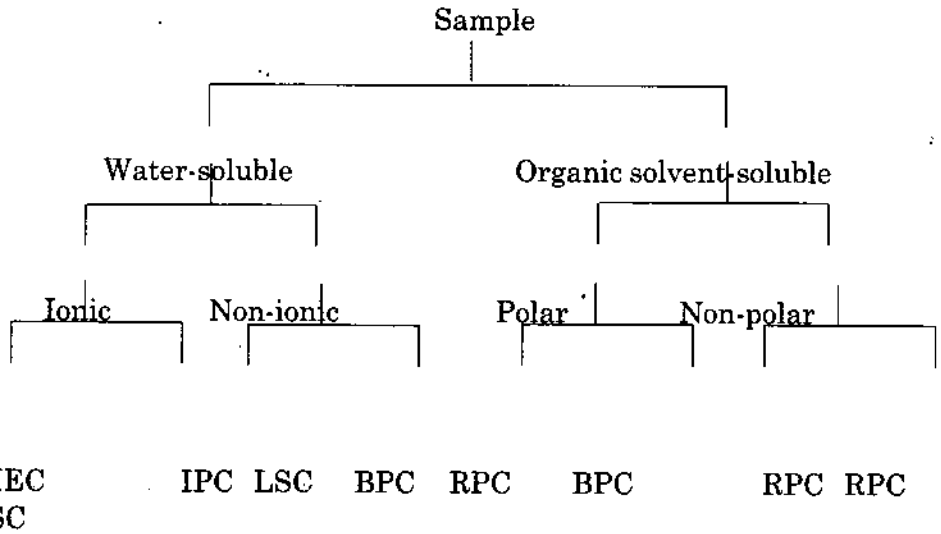
4. Gel permeation (exclusion) chromatography (GPC). This form of liquid chromatography allows the separation of substances according to their molecular size and shape. The stationary phases used in GPC are porous materials with a closely controlled pore size. The primary mechanism of retention of solute molecules is the different penetration by each solute molecule into the interior of the gel particles. Molecules whose size is too great will be effectively barred from certain openings into the gel network and will pass through the column by interstitial liquid volume. Smaller molecules are better able to penetrate into the interior of the gel particles, depending on their size and upon the distribution of pore sizes available to them, and are more strongly retained.

The materials originally used as stationary phases for GPC were the xerogels of the polyacrylamide (Bio-Gel) and cross-linked dextran (Sephadex) type. These semi-rigid gels are unable to withstand the high pressures used in HPLC. Modern stationary phases consist of microparticles of styrene divinylbenzene copolymers (Ultrastyragel, manufactured by Waters Associates), silica, or porous glass.

The analytical applications of GPC cover both organic and inorganic materials. Although there have been many applications of GPC to simple

inorganic and organic molecules, the technique has been mainly applied to studies of complex biochemical or highly polymerised molecules.

Choice of mode of separation. To select the most appropriate column type, the analyst requires some knowledge of the physical characteristics of the sample as well as the type of information required from the analysis. The diagram (below) gives a general guide to the selection of a chromatographic method for separation of compounds of molecular weight < 2000; for samples of higher molecular weight (> 2000) the method of choice would be size-exclusion or gel-permeation chromatography. For a complex sample, no single method may be completely adequate for the separation and a combination of techniques may be required.



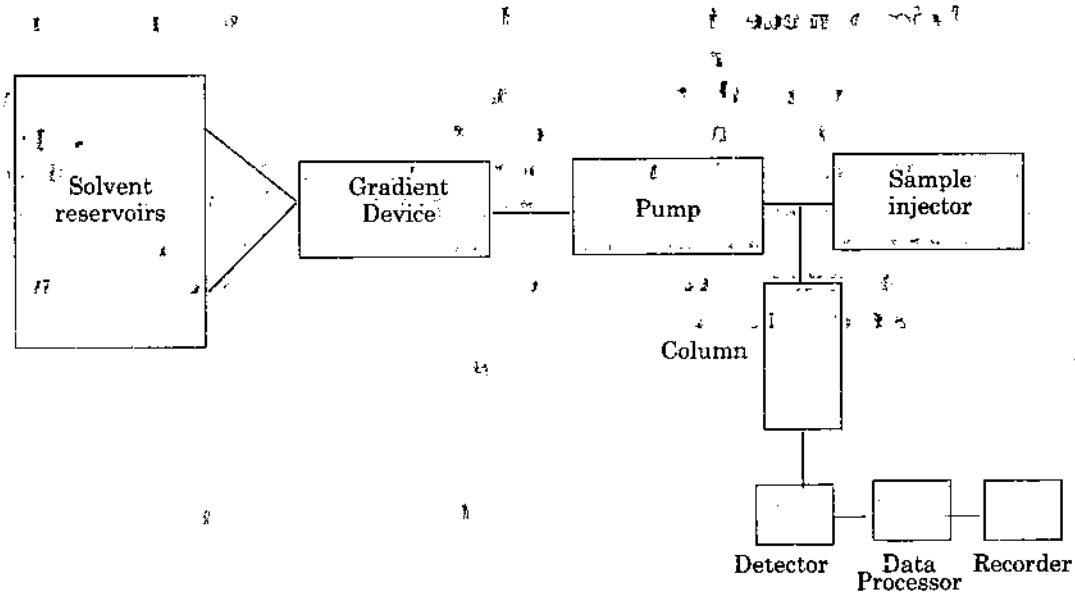
EQUIPMENT FOR HPLC

The essential features of a modern liquid chromatograph are given in the block diagram and consists of the following components :

1. Solvent delivery system which includes a pump, associated pressure and flow controls and a filter on the inlet side;
2. sample injection system;
3. the column;
4. the detector;
5. strip chart recorder;
6. data handling device and microprocessor control.

High-pressure pumps. The pump is one of the most important components of the liquid chromatograph, because its performance directly affects retention time, reproducibility and detector sensitivity. For analytical applications where columns 25–50 cm in length (4.0–10 mm i.d.) and packed with particles as small as 5 or 10 μm are used, the pump should be capable of delivering the mobile phase at flow rates of 1–5 mL min^{-1} and at pressures reaching 5000 psi. Most of the work in analytical HPLC is done using pressures between about 400 and 1500 psi. Although the SI unit of pressure is the Pascal (Pa), instrument manufacturers commonly report pressures in bar or pounds per square inch (psi), the relevant conversion factors being, 1 bar = 10^5 Pa = 14.5 psi. The supply of mobile phase to the column should be constant and pulse-free.

The types of pumps used for HPLC can be divied into two categories : **constant-pressure pumps** (*e.g.* the inexpensive gas-displacement pump) and



Modern Liquid chromatograph

the **constant-volume type** (*e.g.* the reciprocating and syringe pumps). The most commonly used pumps in HPLC are the single- or multi-head reciprocating type. The constant-pressure pump delivers the flow as a series of pulses which must be damped using a pulse dampener; dual- and triple-head constant-volumetype pumps can be operated without a pulse dampener since they minimise pulsation, but are more expensive than single-head pumps.

The choice of a suitable **mobile phase** is vital in HPLC and it is important to refer to the factors influencing this choice. Thus, the eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For 'normal-phase' separations eluting power increases with increasing polarity of the solvent, while for 'reverse-phase' separations eluting power decreases with increasing solvent polarity.

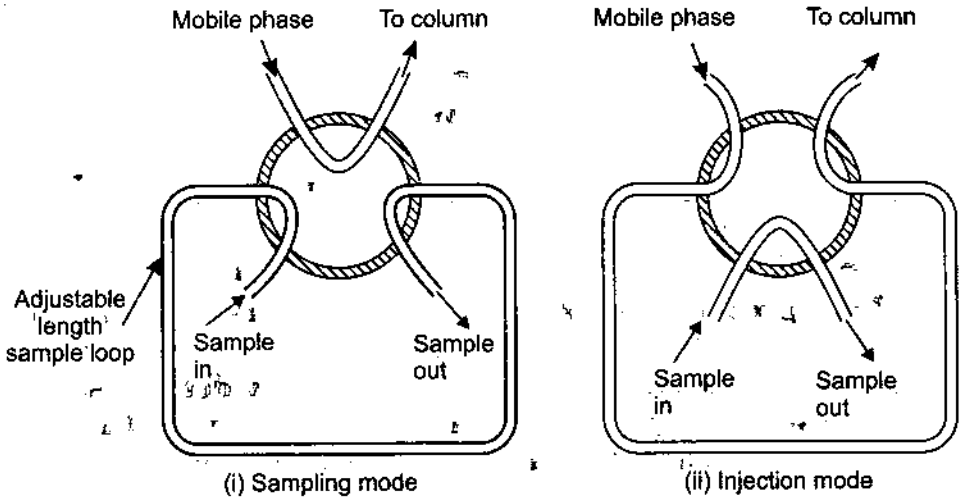
Other properties of solvents which need to be considered are boiling point, **viscosity** (lower viscosity generally gives greater chromatographic efficiency), **detector compatibility, flammability**, and **toxicity**. Many of the common solvents used in HPLC are flammable and some are toxic and it is therefore advisable for HPLC instrumentation to be used in a well-ventilated laboratory, if possible under an extraction duct or hood.

Special grades of solvents are available for HPLC which have been carefully purified to remove UV-absorbing impurities and any particulate matter. If other grades of solvent are used, purification may be required because UV-absorbing impurities may affect the detector and impurities of higher polarity than the solvent (*e.g.* traces of water or ethanol, commonly added as a stabiliser, in chloroform) may influence the separation. It is also important to remove dissolved air or suspended air bubbles which can be a major cause of practical problems in HPLC, particularly affecting the operation of the pump and detector. These problems may be avoided by de-gassing the mobile phase before use.

Sample injection system. Introduction of the sample is generally done either by using syringe injection or through a sampling valve.

Septum injectors allow sample introduction by a high-pressure syringe through a self-sealing elastomer septum. One of the problems found with septum injectors is the leaching effect of the mobile phase in contact with the septum, which may give rise to ghost peaks. Therefore, syringe injection for HPLC is more troublesome.

Although the problems found with septum injectors can be removed by using stop-flow septumless injection as given in diagrams, which enable samples to be introduced into pressurised columns without interruption of the mobile phase flow. The sample is loaded at atmospheric pressure into an external loop in the valve and introduced into the mobile phase by an suitable rotation of the valve. The volume of sample introduced, ranging from 2 μL to over 100 μL, may be varied by changing the volume of the sample loop or by using special variable-volume sample valves. Automatic sample injectors are also available which allow unattended (*e.g.* overnight) operation of the instrument.



Operation of a sample loop.

The column. The columns used are made from precision-bore polished stainless steel tubing, typical dimensions being 10-30 cm long and 4 or 5 mm internal diameter. The stationary phase or packing is retained at each end by thin stainless steel frits with a mesh of 2 μm or less.

The packings used in modern HPLC consist of small, rigid particles having a narrow particle-size distribution. The types of packing may be divided into the following three categories.

(a) **Porous, polymeric beads** based on styrene-divinylbenzene copolymers. These are used for ion exchange and size exclusion chromatography, but have been replaced for many analytical applications by silica-based packings which are more efficient and mechanically stable.

(b) **Porous-layer beads** (diameter 30–55 μm) consisting of a thin shell (1-3 μm) of silica, or modified silica or other material, on an inert spherical core (*e.g.* glass beads). These pellicular-type packings are used for some ion exchange applications, but their general use in HPLC has declined with the development of totally porous microparticulate packings.

(c) **Totally porous silica particles** (diameter < 10 μm, with narrow particle size range) are now the basis of the most commercially important column packings for analytical HPLC. Totally porous silica

particles show improvements in column efficiency, sample capacity, and speed of analysis.

The development of bonded phases for liquid-liquid chromatography on silica-gel columns is of major importance. For example, the widely used C-18 type permits the separation of moderately polar mixtures and is used for the analysis of pharmaceuticals, drugs and pesticides.

The procedure chosen for column packing depends on the mechanical strength of the packing and its particle size. Particles of diameter > 20 μm can be dry-packed, whereas for particles with diameters < 20 μm slurry packing techniques are used in which the particles are suspended in a suitable solvent and the suspension (or slurry) driven into the column under pressure.

The useful life of an analytical column is increased by introducing a **guard column**. This is a short column which is kept between the injector and the HPLC column to protect the latter from damage or loss of efficiency caused by particulate matter or strongly adsorbed substances in samples or solvents. It may also be used to saturate the eluting solvent with soluble stationary phase. Guard columns may be packed with microparticulate stationary phases or with porous-layer beads; and have lower capacities and therefore require changing more frequently.

Detectors. The function of the detector in HPLC is to monitor the mobile phase. The detection process in liquid chromatography has presented more problems. Suitable detectors can be divided into the following two classes :

(a) ***Bulk property detectors*** which measure the difference in some physical property of the solute in the mobile phase compared to the mobile phase alone, *e.g.* **refractive index** and **conductivity detectors**. They are generally universal in application but have poor sensitivity and limited range. Such detectors are usually affected by even small changes in the mobile-phase composition.

(b) ***Solute property detectors*** *e.g.* spectrophotometric, fluorescence and electrochemical detectors. These respond to a particular physical or chemical property of the solute, being ideally independent of the mobile phase. They generally provide high sensitivity (about 1 in 10^9 being attainable with UV and fluorescence detectors) and a wide linear response range but, as a consequence of their more selective natures, more than one detector may be required to meet the demands of an analytical problem.

Some of the important characteristics required of a detector are the following.

(a) ***Sensitivity***, which is often expressed as the noise equivalent concentration, *i.e.* the solute concentration, C_n, which produces a signal equal to the detector noise level. The lower the value of C_n for a particular solute, the more sensitive is the detector for that solute.

(b) ***A linear response***. The linear range of a detector is the concentration range over which its response is directly proportional to the concentration of solute.

(c) ***Type of response***, *i.e.* whether the detector is **universal** or **selective**. A 'universal' detector will sense all the constituents of the sample, whereas a 'selective' detector will only respond to certain components. Although the response of the detector will not be independent of the operating conditions, e.g. column temperature or flow rate, it is advantageous if the

response does not change too much when there are small changes of these conditions.

Different types of detectors are given below :

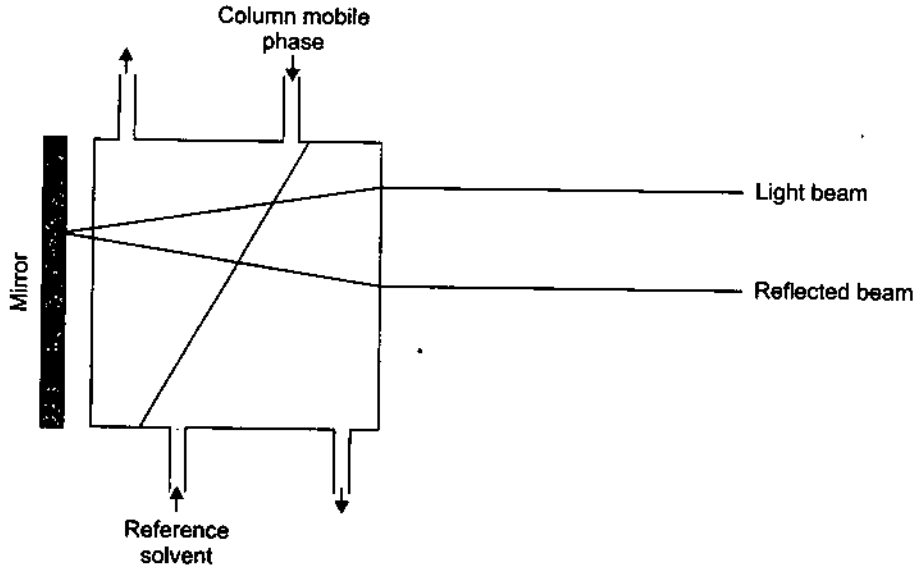
Detector in HPLC

Type	Response	C_n (g mL^{-1})
Amperometric	Selective	10^{-10}
Conductimetric	Selective	10^{-7}
Fluorescence	Selective	10^{-12}
UV/visible absorption	Selective	10^{-8}
Refractive index	Universal	10^{-6}

A brief account of the main types of detectors is given below :

1. Refractive index detectors. These bulk property detectors are based on the change of refractive index of the eluant from the column with respect to pure mobile phase. Although they are widely used, the refractive index detectors suffer from several disadvantages : **lack of high sensitivity, lack of suitability for gradient elution,** and the **need for strict temperature control** (± 0.001 °C) to operate at their highest sensitivity. A pulseless pump, or a reciprocating pump equipped with a pulse dampener, must also be used. The effect of these limitations may overcome by the use of differential systems in which the column eluant is compared with a reference flow of pure mobile phase. The two main types of RI detector are as follows :

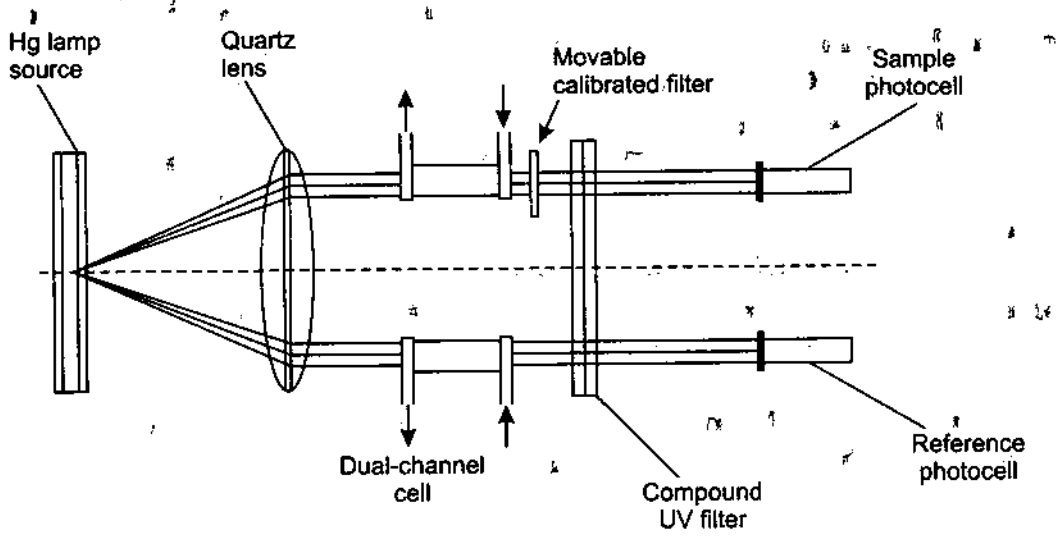
(i) ***The deflection refractometer.*** It measures the deflection of a beam of monochromatic light by a double prism in which the reference and sample cells are separated by a diagonal glass divide. When both cells contain solvent of the same composition, no deflection of the light beam occurs; if, the composition of the column mobile phase is changed because of the presence of a solute, then the altered refractive index causes the beam to be deflected. The magnitude of this deflection is dependent on the concentration of the solute in the mobile phase.



(ii) ***The Fresnel refractometer.*** It measures the change in the fractions of reflected and transmitted light at a glass-liquid interface as the refractive

index of the liquid changes. In this detector, both the column mobile phase and a reference flow of solvent are passed through small cells on the back surface of a prism. When the two liquids are identical there is no difference between the two beams reaching the photocell, but when the mobile phase containing solute passes through the cell there is a change in the amount of light transmitted to the photocell, and a signal is produced. The smaller cell volume (about 3 μL) in this detector makes it more suitable for high-efficiency columns but, for sensitive operation, the cell windows must be kept scrupulously clean.

2. Ultraviolet detectors. The UV absorption detector is mostly used in HPLC. It is based on the principle of absorption of UV light as the effluent from the column is passed through a small flow cell held in the radiation beam. It is characterised by high sensitivity (detection limit of about 1×10^{-9} g mL^{-1} for highly absorbing compounds) and, as it is a solute property detector, it is relatively insensitive to changes of temperature and flow rate. The detector is suitable for gradient elution work. The presence of air bubbles in the mobile phase can greatly impair the detector signal, causing spikes on the chromatogram. This effect can be minimised by degassing the mobile phase.



3. Diode array (multichannel) detector. In which polychromatic light is passed through the flow cell. The emerging radiation is diffracted by a grating and then falls on to an array of photodiodes, each photodiode receiving a different narrow wavelength band. A microprocessor scans the array of diodes many times a second and the spectrum so obtained may be stored in the instrument for subsequent print-out. An important feature of the multichannel detector is that it can be programmed to give changes in detection wavelength at specified points in the chromatogram.

4. Fluorescence detectors : These devices enable fluorescent compounds (solutes) present in the mobile phase to be detected by passing the column effluent through a cell irradiated with ultraviolet light and measuring any resultant fluorescent radiation. Although only a small proportion of inorganic and organic compounds are naturally fluorescent, many biologically active compounds (*e.g.* drugs) and environmental contaminants (*e.g.* polycyclic aromatic hydrocarbons) are fluorescent which, together with the high sensitivity of these detectors, explains their widespread use.

5. Electrochemical detectors : The term 'electrochemical detector' in HPLC normally means to amperometric or coulometric detectors, which

measure the current associated with the oxidation or reduction of solutes. It is difficult to use electrochemical reduction as a means of detection in HPLC because of the serious interference (large background current) caused by reduction of oxygen in the mobile phase. Complete removal of oxygen is difficult so that electrochemical detection is usually based on oxidation of the solute. Compounds which can be conveniently detected in this way are phenols, aromatic amines, heterocyclic nitrogen compounds, ketones, and aldehydes. Electrochemical detection requires the use of conducting mobile phases, *e.g.* containing inorganic salts or mixtures of water with water-miscible organic solvents.

The amperometric detector is most widely used electrochemical detector, having the advantages of high sensitivity and very small internal cell volume. Three electrodes are used in these detectors, which are given as :

(a) The working electrode, commonly made of glassy carbon. It is the electrode at which the electroactive solute species is monitored ;

(b) The reference electrode, generally a silver-silver chloride electrode. It gives a stable, reproducible voltage to which the potential of the working electrode is referred;

(c) The auxiliary electrode. It is the current-carrying electrode and made of stainless steel.

Despite the higher sensitivity, and relative cheapness compared with ultraviolet detectors, amperometric detectors have a more limited range of applications, and often used for trace analyses where the ultraviolet detector does not have sufficient sensitivity.

DERIVATISATION

In liquid chromatography derivatives are almost invariably prepared to enhance the response of a particular detector to the substance of analytical interest. For example, with compounds lacking an ultraviolet chromophore in the 254 nm region but having a reactive functional group, derivatisation provides a means of introducing into the molecule a chromophore suitable for its detection. Derivative preparation can be carried out either prior to the separation (pre-column off line derivatisation) or afterwards (post-column on-line derivatisation).

1. Pre-column off-line derivatisation requires no modification to the instrument. Its disadvantages are that the presence of excess reagent and by-products may interfere with the separation, while the group introduced into the molecules may change the chromatographic properties of the sample.

2. Post-column on-line derivatisation is carried out in a special reactor situated between the column and detector. A feature of this technique is that the derivatisation reaction need not go to completion. The reaction needs to be rapid at moderate temperatures and there should be no detector response to any excess reagent present. An advantage of post-column derivatisation is that ideally the separation and detection processes can be optimised separately. A problem which may arise is that the most suitable eluant for the chromatographic separation rarely provides an ideal reaction medium for derivatisation. This is true for electrochemical detectors which operate only within a limited range of pH, ionic strength and aqueous solvent composition.

Reagents which form a derivative that strongly absorbs UV/visible radiation are called **chromatags;** For example the reagent ninhydrin, commonly used to obtain derivatives of amino acids which show absorption at about 570 nm. Derivatisation for fluorescence detectors is based on the reaction of nonfluorescent reagent molecules **(fluorotags)** with solutes to form fluorescent derivatives; the reagent dansyl chloride (I) is used to obtain fluorescent derivatives of proteins, amines and phenolic compounds,

QUANTITATIVE ANALYSIS

Quantitative analysis by HPLC requires that a relationship is established between the magnitude of the detector signal and the concentration of a solute in the sample. The magnitude can be measured by either the corresponding peak area or the peak height. Peak area measurements are done when the column flow can be controlled precisely, since peak area is relatively independent of mobile-phase composition. Manual methods may be used for calculating peak areas but computing integrators are preferred for data handling in chromatography and are now often a part of the instrumental package. If the peak areas are measured with an integrator, the latter prints out the retention time for each peak with a number which is proportional to the peak area. The percentage of each compound in the mixture may then be calculated on the basis of area normalisation, *i.e,* by expressing each peak area as a percentage of the total area of all the peaks in the chromatogram. As the detector response is likely to differ for the various components of the mixture, it is essential to correct each peak area before using area normalisation; this is done by finding the relative response factors for the detector.

THIN-LAYER CHROMATOGRAPHY

The important difference between thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) is one of practical technique rather than of the physical phenomena (adsorption, partition, etc.) on which separation is based. In TLC the stationary phase consists of a thin layer of sorbent (e.g. silica gel or cellulose powder) coated on an inert, rigid, packing material such as a glass plate or plastic foil so that the separation process occurs on a flat and two-dimensional surface. Although TLC is widely used for qualitative analysis, it does not provide quantitative information of high precision and accuracy. Recent changes in the practice of TLC have resulted in improved performance both in terms of separations and in quantitative measurements. These developments are referred to as high-performance thin-layer chromatography (HPTLC).

Technique of thin-layer chromatography : ***Preparation of the plate.*** In thin-layer chromatography a variety of coating materials is available, but silica gel is most commonly used. A slurry of the adsorbent (silica gel, cellulose powder, etc.) is spread uniformly over the plate by means of spreader, the recommended thickness of adsorbent layer being 150-250 μm. After air-drying overnight, or oven-drying at 80-90°C for about 30 minutes, plate is ready for use.

Thin-layers (*i.e.* pre-coated plates or plastic sheets) are commercially available; the main advantage of plastic sheets is that they can be cut to any size or shape required, but they have the disadvantage that they bend in the chromatographic tank unless supported.

Regarding TLC two points of practical precautions must be kept in mind :

1. care should be taken in handling the plate to avoid placing fingers on the active adsorbent surface,

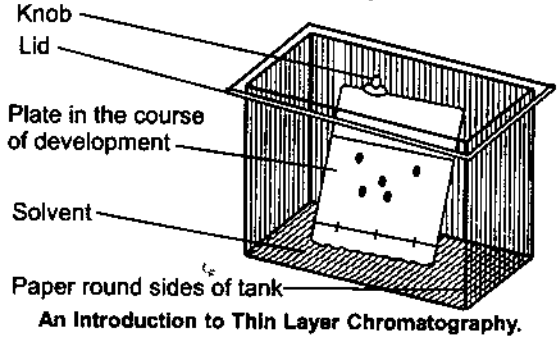
2. pre-washing of the plate is advisable to remove any impurities contained in the layer, and this may be done by running the development solvent to the top of the plate.

Sample application : The origin line, to which the sample solution is applied, located 2-2.5 cm from the bottom of the plate. Volumes of 1, 2 or 5 μL are applied using an appropriate measuring instrument, *e.g.* an Agla syringe or a Drummond micropipette (the latter is a calibrated capillary tube fitted with a small rubber teat). Care must be taken to avoid disturbing the surface of the adsorbent as this causes distorted shapes of the spots on the developed chromatogram and so hinders quantitative measurement.

Development of plates : The chromatogram is developed by the ascending technique in which the plate is immersed in the developing solvent to a depth of 0.5 cm. The tank or chamber used is lined with sheets of filter paper which dip into the solvent in the base of the chamber; this ensures that the chamber is saturated with solvent vapour. Development is allowed to proceed until the solvent front has travelled the required distance (usually 10-15 cm), the plate is then removed from the chamber and the solvent front immediately marked with a pointed object.

The plate is allowed to dry in a fume cupboard or in an oven.

The positions of the separated solutes can be located by various methods. Coloured substances can be seen directly while colourless species may be detected by spraying the plate with an appropriate reagent which produces coloured areas in the regions. Some compounds fluoresce in ultraviolet light and may be located in this way. Alternatively if fluorescing materials are mixed in the adsorbent the solute can be observed as a dark spot on a fluorescent background when viewed under ultraviolet light. The spots located by this method can be delineated by marking with a needle.



An Introduction to Thin Layer Chromatography.

Quantitative evaluation. Methods for the quantitative measurement of separated solutes on a thin-layer chromatogram can be divided into two categories. (1) By densitometer (2) By reflectance or absorbtion of light beam. The latter instrument scans the individual spots. The scan usually being along the line of development of the plate. The difference in intensity of the reflected (or transmitted) light between the adsorbent and the solute spots is observed

as a series of peaks plotted by a chart recorder. The areas of the peaks correspond to the quantities of the substances in the various spots. This type of procedure requires comparison with spots obtained using known amounts of standard mixtures which must be chromatographed on the same plate as the sample. In densitometer method, the photodensity of the spots is measured directly on the thin-layers plate.

The alternative, and cheaper, procedure is to remove the separated components by scraping off the relevant portion of the adsorbent after visualisation by a non-destructive technique. The component is extracted by placing the adsorbent in a centrifuge tube and adding a suitable solvent to dissolve the solute. When the solute has dissolved the tube is spun in a centrifuge, the supernatant liquid removed and analysed by an suitable quantitative technique, *e.g.* ultraviolet, visible or fluorescence spectrometry or gas-liquid chromatography. Alternatively the solute may be extracted by transferring the adsorbent on to a short column of silica gel supported by a sinter filter and eluting with the solvent. Again the extract is analysed by a suitable quantitative technique. In each case, it is necessary to obtain a calibration curve for known quantities of the solute in the chosen solvent.

To obtain the best results in any of these quantitative TLC methods, the spots being used should have R_f values between 0.3 and 0.7; spots with R_f values < 0.3 end to be too concentrated whereas those with R_f values > 0.7 are too diffuse.

HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

Recent developments in the thin-layer chromatography have resulted in a breakthrough in performance which has led to the expression 'high performance thin-layer chromatography'. The three main features of HPTLC are summarised below:

1. Quality of the adsorbent layer : Thin layers for HPTLC are prepared using purified silica gel with average particle diameter of 5-15 μm and a narrow particle size distribution. The silica gel may be modified if necessary, e.g. chemically bonded layers are available commercially as reverse-phase plates. Layers prepared using these improved adsorbents, this enables more difficult separations to be effected using HPTLC, and also enables separations to be achieved in much shorter times.

2. Methods of sample application : Due to the lower sample capacity of the HPTLC layer, the required amount of sample applied to the layer is reduced. Sample volumes are 100-200 nL which give spots of only 1.0-1.5 mm diameter; after developing the plate for a distance of 3-6 cm. compact separated spots are obtained which gives detection limits about ten times better than in conventional TLC. A further advantage is that the compact starting spots allow an increase in the number of samples which may be applied to the HPTLC plate.

The introduction of the sample into the adsorbent layer is a critical process in HPTLC. For most quantitative work a platinum iridium capillary of fixed volume (100 or 200 nL), sealed into a glass support capillary of larger bore, provides a convenient spotting device. The capillary tip is polished to provide a smooth, planar surface of small area (*ca* 0.05 mm^2), which when used with a mechanical applicator minimises damage to the surface of the plate. Spotting by manual procedures damages the surface.

3. The availability of scanning densitometers. Although double beam instruments are available, single beam single wavelength operation is mainly used in HPTLC. The quality and surface homogeneity of the plates are generally very good.

High performance thin-layer chromatography has found its application in the areas of clinical (*e.g.* analysis of drugs in blood) and environmental analysis.

DETERMINATION OF ASPIRIN, PHENACETIN AND CAFFEINE IN A MIXTURE

High performance liquid chromatography (HPLC) is used for the separation and quantitative analysis of a variety of mixtures, especially those in which the components are insufficiently volatile and thermally stable. The following method which may be used for the quantitative determination of aspirin and caffeine in the common analgesic tablets, using phenacetin as internal standard; where APC tablets are available the phenacetin can also be determined by this procedure.

Sample mixture. A sample mixture is obtained by weighing out accurately 0.601 g of aspirin, 0.076 g of phenacetin and 0.092 g of caffeine. Dissolve the mixture in 10 mL absolute ethanol, add 10 mL of 0.5M ammonium formate solution and dilute to 100 mL with de-ionised water.

Solvent (mobile phase). Ammonium formate (0.05*M*) in 10 per cent (*v* / *v*) ethanol-water at pH 4.8. Use a flow rate of 2 mL min^{-1} with inlet pressure of about 117 bar (1 bar = 10^5 Pa).

Column. 15.0 cm × 4.6 mm, packed with a 5 μm silica SCX (strong cation exchanger) bonded phase.

Detector. UV absorbance at 244 nm

Procedure. Inject 1 μL of the sample solution and obtain a chromatogram. Compounds are separated in about 3 minutes, the elution sequence being (1) aspirin; (2) phenacetin; (3) caffeine. Measure peak areas with an integrator and normalise the peak area for each compound (i.e. express each peak area as a percentage of the total peak area). Compare these results with the known composition of the mixture.

Determine the response factors (*r*) for the detector relative to phenacetin (= 1) as internal standard by carrying out three runs, using 1 μL injection, and obtaining the average value of *r*.

$$\text{Relative response factor, } r = \frac{\text{Peak area of compound / Mass of compound}}{\text{Peak area of standard / Mass of standard}}$$

Correct the peak areas initially obtained by dividing by the suitable response factor and normalise the corrected values. Compare this result with the known composition of the mixture.

GAS CHROMATOGRAPHY

INTRODUCTION

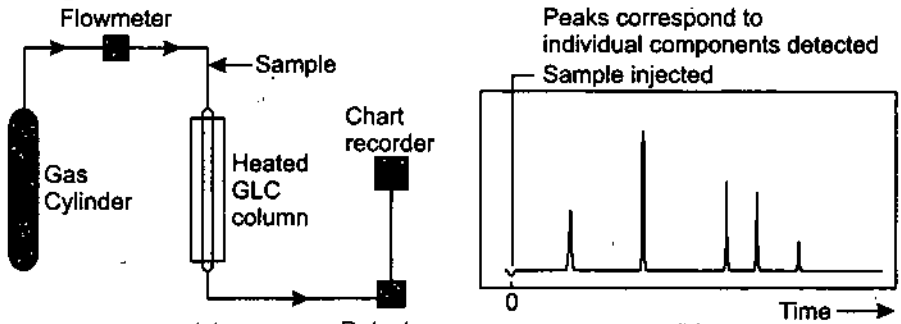
Gas chromatography is a process by which a mixture is separated into its constituents by a moving gas phase passing over a stationary sorbent. The technique is similar to liquid-liquid chromatography except that the mobile liquid phase is replaced by a moving gas phase. Gas chromatography is divided into two major categories; **gas-liquid chromatography** (GLC), where separation occurs by partitioning a sample between a mobile gas phase and a

thin layer of non-volatile liquid coated on an inert support, and **gas-solid chromatography** (GSC), which employs a solid of large surface area as the stationary phase.

APPARATUS

A gas chromatograph consists of the following parts :

1. A supply of carrier gas from a high-pressure cylinder : The carrier gas used is either helium, nitrogen, hydrogen or argon, the choice of gas depending on factors such as availability, purity required, consumption and the type of detector used. Thus helium is preferred when thermal conductivity detectors are used because of its high thermal conductivity. Associated with this high pressure supply of carrier gas are the attendant pressure regulators and flow meters to control and monitor the carrier gas flow; the operating efficiency of the apparatus is dependent on the maintenance of a constant flow of carrier gas.



Intruments of Gas chromatograph **Typical chart Record of Gas Chromatograph**

The following precautions should be kept in considerations :

(a) free-standing gas cylinders must always be supported by means of clamps or chains;

(b) waste gases, especially hydrogen, must be vented through an extraction hood.

2. Sample injection system and derivatisation. The major applications involve liquid samples which are introduced using a microsyringe with hypodermic needle. The needle is inserted through a self-sealing silicone rubber septum and the sample injected smoothly into a heated metal block at the head of the column. The aim must be to introduce the sample in a reproducible manner. The temperature of the sample port should be such that the liquid is rapidly vaporised but without either decomposing or fractionating the sample; a useful rule of thumb is to set the sample port temperature approximately to the boiling point of the least volatile component. For greatest efficiency, the smallest possible sample size ($1 - 10\ \mu L$) consistent with detector sensitivity should be used.

Many samples are unsuitable for direct injection into a gas chromatograph because of their high polarity, low volatility or thermal instability. In this respect the versatility and application of gas chromatography has been greatly extended by the formation of volatile derivatives, especially by the use of silylation reagents. The term 'silylation' is normally taken to mean the

introduction of the trimethylsilyl, / $Si(CH_3)_3$, or similar group in place of active hydrogen atoms in the substance under investigation. A number of such reagents is now available, including some special silylating agents which give improved detector response, by incorporating a functional group suitable for a selective detector system. Reagents containing chlorine and bromine atoms in the silyl group are used particularly for preparing derivatives injected on to gas chromatographs fitted with electron-capture detectors. Derivatisation can also give enhanced resolution from other components in a mixture and improved peak shape for quantitative analysis.

Although inorganic compounds are generally not so volatile as are organic compounds, gas chromatography has been applied in the study of certain inorganic compounds which possess the requisite properties. If gas chromatography is to be used for metal separation and quantitative analysis, the types of compounds which can be used are limited to those that can be readily formed in quantitative and easily reproducible yield.

The number of applications to analytical determinations at the trace level appear to be few, probably the best known being the determination of beryllium in various samples. The method generally involves the formation of the volatile beryllium trifluoroacetylacetonate chelate, its solvent extraction into benzene with subsequent separation and analysis by gas chromatography.

Various types of derivatisation have now been developed for both gas and liquid chromatography.

For compounds of high molecular mass the formation of derivatives does not help to solve the problem of involatility. This difficulty may be overcome by breaking the large molecules up into smaller and more volatile fragments which may then be analysed by gas-liquid chromatography, *i.e.* by using the technique known as **pyrolysis gas chromatography (PGC).**

Pyrolysis GC is a technique in which a non-volatile sample is pyrolysed under rigidly controlled conditions, usually in the absence of oxygen, and the decomposition products separated in the gas chromatographic column. The resulting chromatogram (pyrogram) is used for both qualitative and quantitative analysis of the sample. If the latter is very complex, complete identification of the pyrolysis fragments may not be possible, but in such cases the pyrogram may be used to 'fingerprint' the sample. PGC has been applied to a wide variety of samples, but its major used has been in polymer analysis for the investigation of both synthetic and naturally occurring polymers. The various PGC systems can be classified into two types :

(a) *Static-mode (furnace) reactors* which consist of a quartz reactor tube and a Pregl type of combustion furnace. Solid samples are placed in the reactor tube and the system is closed. The furnace is then placed over the combustion tube and the sample heated to the pyrolysis temperature in this type of

pyrolysis system, the time required to reach the necessary temperature is much longer (up to 30 seconds) than in dynamic pyrolysis resulting in a greater number of secondary reactions. An important advantage of the static-mode system is the larger sample capacity.

(b) *Dynamic (filament) reactors* in which the sample is placed on the tip of a filament or wire igniter (platinum and nichrome wires have been used) which is then sealed in a reactor chamber; in PGC the latter is the injection port of the gas chromatograph. As the carrier gas passes over the sample, a d.c. charge is applied, and the sample is heated rapidly to the pyrolysis temperature. As the sample decomposes, the pyrolysis products are carried away into a cooler area before entering the gas chromatographic column.

3. The column. The actual separation of sample components is effected in the column where the nature of the solid support, type and amount of liquid phase, method of packing, length and temperature are important factors in obaining the desired resolution.

The column is enclosed in a thermostatically controlled oven so that its temperature is held constant to within 0.5°C, thus ensuring reproducible conditions. The operating temperature may range from ambient to over 400°C and for isothermal operation is kept constant during the separation process.

Although many types of column have been developed for gas chromatography, they may be divided into two major groups :

(a) Packed columns. Conventional analytical columns are usually prepared with 2-6 mm internal diameter glass tubing or 3-10 mm outer diameter metal tubing, which is normally coiled for compactness. Glass columns must be used if any of the sample components are decomposed by contact with metal.

The material chosen as the inert support should be of uniform granular size, and have good handling characteristics and be capable of being packed into a uniform bed in a column. The surface area of the material should be large so that to promote distribution of the liquid phase as a film and ensure the rapid attainment of equilibrium between the stationary and mobile phases. The most commonly used supports (*e.g.* Celite) are made from diatomaceous materials which can hold liquid phases in amounts exceeding 20 per cent without becoming too sticky to flow freely and can be easily packed.

These supports are available in narrow mesh-range fractions, to obtain particles of uniform size the material should be sieved to the desired particle size range and repeatedly water floated to remove fine particles which contribute to excessive pressure drop in the final column. To a good approximation the height equivalent of a theoretical plate is proportional to the average particle diameter so that the smallest possible particles should be preferred in terms of column efficiency. Decreasing particle size will rapidly

increase the gas pressure necessary to achieve flow through the column and in practice the best choice is 80/100 mesh for a 3 mm i.d. column. For effective packing of any column, the internal diameter of the tubing should be at least eight times the diameter of the solid support particles.

Various types of **porous polymers** have also been developed as column packing material for gas chromatography, which are *e.g.* styrene copolymers although modified with different polar, monomers. An entirely different type of porous polymer is Tenax GC which is based on 2,6-diphenylphenylene oxide. A special feature of this column packing is its high maximum operating temperature of 400°C. Tenax GC has been used for concentrating and determining trace volatile organic constituents in gases and biological fluids.

The selection of the most suitable liquid phase for a particular separation is important. Liquid phases can be classified as follows :

1. Non-polar hydrocarbon-type liquid phases, *e.g.* paraffin oil (Nujol), squalane. Apiezon L grease and silicone-gum rubber; the latter is used for high-temperature work (upper limit ~ 400°C).

2. Compounds of intermediate polarity which have a polar group attached to a large non-polar skeleton, *e.g.* esters of high-molecular-weight alcohols such as dinonyl phthalate.

3. Polar compounds containing large proportion of polar groups. e.g. the carbowaxes (polyglycols).

4. Hydrogen-bonding class, *i.e.* polar liquid phases such as glycol, glycerol. hydroxyacids, etc., which contain hydrogen atoms available for hydrogen bonding.

The column packing is prepared by adding the correct amount of liquid phase dissolved in a suitable solvent (*e.g.* acetone or dichloromethane) to a weighed quantity of the solid support in a suitable dish. The volatile solvent is removed by spontaneous evaporation or by careful heating, the mixture gently agitated to ensure a uniform distribution of the liquid phase in the support. Final traces of the solvent may be removed under vacuum and the column packing re-sieved to remove any fines produced during the preparation. The amount of stationary liquid phase in the column packing is expressed on the basis of the percentage by weight of liquid phase present. e.g. 15 per cent loading indicates that 100 g column packing contains 15 g of liquid phase on 85 g of inert support. The solid should remain free flowing after being coated with the liquid phase.

Micropacked columns, referred to as packed capillary columns, have been used in gas chromatography, for the determination of trace components in complex mixtures. These columns are characterised by small internal diameters (i.d. < 1.0 mm) and packing densities comparable with conventional packed columns. The column packing technique requires higher pressures and

constant vibration (*e.g.* ultrasonic) to achieve the necessary packing density. Micropacked columns give high efficiency but practical problems, especially sample injection at high back-pressures, have limited their use.

(b) Open tubular columns. These capillary columns (*i.d.* < 1 mm) are used in GLC because of their superior resolving power for complex mixtures. In these capillary columns, the stationary phase is coated on the inner wall of the tube. Two basic types of capillary column are available:

1. **Wall-coated open tubular** (WCOT), in which the stationary phase is coated directly on to the inner wall of the tubing :

2. **Support-coated open tubular** (SCOT), which have a finely-divided layer of solid support material deposited on the inner wall on to which the stationary phase is then coated. These SCOT columns are not as efficient as WCOT columns but have a higher sample capacity.

Capillary columns are fabricated from thin-walled stainless steel, glass, or high-purity fused silica tubing. Typical dimensions of the columns, which are coiled, are 25-200 m long and 0.2-0.5 mm i.d.

Open tubular columns provide a number of stationary phases of differing polarity on WCOT and SCOT columns, and whose efficiency, greatly improved sample detectability, and thermal stability.

4. The detector. The function of the detector, which is situated at the exit of the separation column, is to measure the small amounts of the separated components present in the carrier gas stream leaving the column. The output from the detector is fed to a recorder which produces a pen-trace called a **chromatogram**. The choice of detector depend on factors such as the concentration level to be measured and the nature of the separated components. The detectors most widely used in gas chromatography are the **thermal conductivity, flame-ionisation** and **electron-capture detectors.**

Some of the important properties of a detector in gas chromatography are given below :

(a) **Sensitivity**. This is defined as the detector response (mV) per unit concentration of analyte (mg mL^{-1}). It is closely related to the limit of detection (MDL) as high sensitivity often gives a low limit of detection. The latter is generally defined as the amount of analyte which produces a signal equal to twice the baseline noise, the limit of detection will be raised if the detector produces excessive noise. The sensitivity also determines the slope of the calibration graph and therefore influences the precision of the analysis.

(b) **Linearity**. The linear range of a detector refers to the concentration range over which the signal is directly proportional to the amount of analyte. Linearity in detector response gives linearity of the calibration graph and

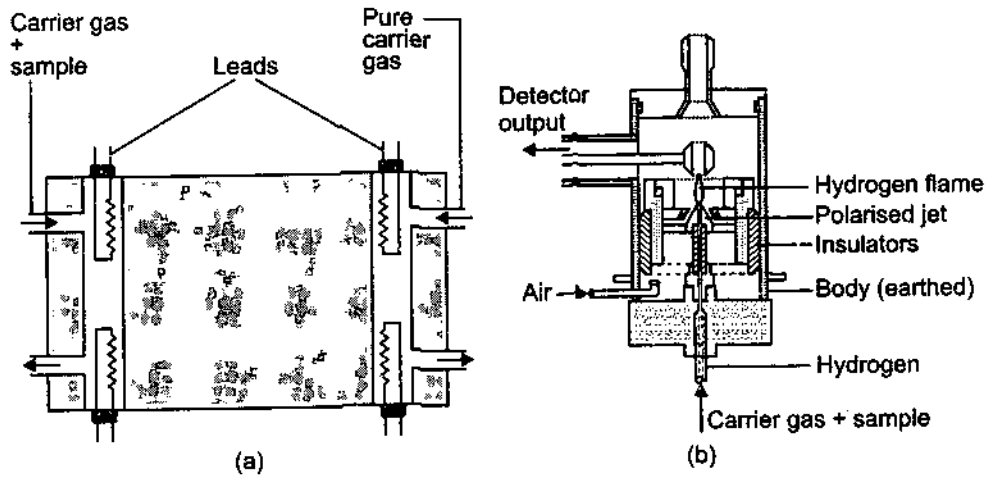
allows the latter to be drawn with more certainty. With a convex calibration curve, the precision is reduced at the higher concentrations where the slope of the curve is much less.

(c) **Stability.** An important characteristic of a detector is the signal output which remains constant with time, assuming there is a constant input. Lack of stability can be exhibited in two ways, *i.e.* by **baseline noise** or by **drift,** both of which will limit the sensitivity of the detector. Baseline noise, caused by a rapid variation in detector output, which makes it difficult to measure small peaks against the fluctuating background. Baseline drift, a slow systematic variation in output, results in a sloping baseline which in many cases may go off scale during the analysis. Drift is often due to factors external to the detector, such as temperature change or column bleed, and so is controllable, whereas noise is usually due to poor contacts within the detector.

(d) **Universal or selective response.** A universal detector will respond to all the components present in a mixture, whereas a selective detector senses only certain components in a sample which can be advantageous if it responds only to those which are of interest, thus giving a simplified chromatogram and avoiding interference.

Thermal conductivity detector. The most important of the bulk physical property detectors is the thermal conductivity detector (TCD) which is universal, non-destructive, concentration-sensitive detector. The TCD was one of the earliest routine detectors. Thermal conductivity cells or katharometers are still used in gas chromatography. These detectors contain a heated metal filament or a thermistor sense changes in the thermal conductivity of the carrier gas stream. Helium and hydrogen are the best carrier gases to use in this type of detector since their thermal conductivities are much higher than any other gases. On safety grounds helium is preferred because of its inertness.

In the detector two pairs of filaments are arranged in a Wheatstone bridge circuit; two filaments in opposite arms of the bridge are surrounded by the carrier gas only, while the other two filaments are surrounded by the effluent from the chromatographic column. Thermal conductivity, cell is given below which have two gas channels through the cell : a sample channel and a reference channel. When pure carrier gas passes over both the reference and sample filaments the bridge is balanced, but when a vapour enters from the column, the rate of cooling of the sample filaments changes and the bridge becomes unbalanced. This imbalance is a measure of the concentration of vapour in the carrier gas and the out-of-balance signal is fed to a recorder thus producing the chromatogram. The differential technique used is thus based on the measurement of the difference in thermal conductivity between the carrier gas and the carrier gas/sample mixture.



(a) Thermal conductivity detector. (b) Flame ionisation detector.

The TCD is used for the detection of permanent gases, light hydrocarbons and compounds which respond poorly to the flame-ionisation detector (FID). For many general applications, it has been replaced by the FID, which is more sensitive (up to 1000-fold), has a greater linear response range, and provides a more reliable signal for quantitative analysis.

Ionisation detectors. An important characteristic of the common carrier gases is that they behave as perfect insulators at normal temperatures and pressures. The increased conductivity due to the presence of a few charged-molecules in the effluent from the column which provides the high sensitivity, it is a main feature of the ionisation based detectors. Ionisation detectors in current use include the flame ionisation detector (FID), thermionic ionisation detector (TID), photoionisation detector (PID) and electron capture detector (ECD) each employing a different method to generate an ion current. The two most widely used ionisation detectors are the FID and ECD.

Flame ionisation detector. The basis of this detector is that the effluent from the column is mixed with hydrogen and burned in air to produce a flame which has sufficient energy to ionise solute molecules having low ionisation potentials. The ions produced are collected at electrodes and the resulting ion current measured. The burner jet is the negative electrode while the anode is usually a wire or grid extending into the tip of the flame. This detector is shown above.

The combustion of mixtures of hydrogen and air produces very few ions so that with only the carrier gas and hydrogen burning an essentially constant signal is obtained. When carbon-containing compounds are present ionisation occurs and there is a large increase in the electrical conductivity of the flame. As the sample is destroyed in the flame a stream-splitting device is used. This device is inserted between the column and detector and allows the bulk of the sample to by-pass the detector.

The FID has wide applicability, it is a very nearly universal detector for gas chromatography of organic compounds, and this, coupled with its high sensitivity, stability, fast response and wide linear response range (~ 10^7). It is the most popular detector in current use.

Electron capture detector. Most ionisation detectors are based on measurement of the increase in current which occurs when a more readily ionised molecule appears in the gas stream. The electron capture detector differs from other ionisation detectors in that it exploits the recombination phenomenon, which is based on electron capture by compounds having an affinity for free electrons. The detector thus measures a decrease rather than an increase in current.

A β-ray source (commonly a foil containing ^3H or ^{63}Ni) is used to generate 'slow' electrons by ionisation of the carrier gas flowing through the detector. These slow electrons migrate to the anode under a fixed potential and give rise to a steady baseline current. When an electron-capturing gas enters from the column and reacts with an electron, the net result is the replacement of an electron by a negative ion of much greater mass with a corresponding reduction in current flow.

The response of the detector is related to the electron affinity of the eluate molecules particularly sensitive to compounds containing halogens and sulphur, anhydrides, conjugated carbonyls, nitrites, nitrates and organometallic compounds. The ECD is the second most widely used ionisation detector due to its high sensitivity to a wide range of compounds. It is used in trace analysis of pesticides, herbicides, drugs, and other biologically active compounds, and detecting the ultratrace amounts of metals as their chelate complexes.

The ECD is chosen for its high selectivity which can simplify chromatograms. The ECD requires careful attention to obtain reliable results such as cleanliness and the carrier gases must be very pure and dry.

A summary of some important detector characteristics are given below :

Detector characteristics

Type	*MDL* ($g\ s^{-1}$)*	*Linear range*	*Temp. limit (°C)*	*Features*
TCD	$10^{-6} - 10^{-8}$	10^4	450	Non-destructive, but temperature and flow-sensitive
FID	10^{-11}	10^7	400	Destructive, excellent stability and linearity
ECD	10^{-13}	10^2	350	Non-destructive but easily contaminated and temperature-sensitive

*The minimum detectable level is given in terms of the mass flow rate in grams per second.

Element-selective detectors. Many samples, which originating from environmental studies, contain so many constituent compounds that the gas chromatogram obtained is a complex array of peaks. For the analytical chemist, who may be interested in only a few of the compounds present, the replacement of the non-selective type of detector (i.e. thermal conductivity, flame ionisation, etc.) by a system which responds selectively to some property of certain of the eluted species may overcome this problem.

The selective detectors generally respond to the presence of a characteristic element or group in the eluted compound. This is well illustrated by the thermionic ionisation detector (TID) which is a flame ionisation detector giving a selective response to phosphorus and /or nitrogen-containing compounds. The TID contains an electrically heated rubidium silicate bead situated a few millimetres above the detector jet tip and below the collector electrode. The temperature of the bead is maintained at 600-800°C while a plasma is introduced in the region of the bead by hydrogen and air support gases. A reaction cycle is so produced in which the rubidium is vaporised, ionised and finally recaptured by the bead. During this process an electron flow to the positive collector electrode occurs and this background current is enhanced when nitrogen or phosphorus compounds are eluted, it is thought to the production of radicals in the flame or plasma which accelerate the rate of rubidium recycling.

Another example is the flame photometric detector (FPD) which offers Simultaneous sensitivity and specificity for the determination of compounds containing sulphur and phosphorus. The operating principle of the FPD is that combustion of samples conaining phosphorus or sulphur in a hydrogen-rich flame results in the formation of luminescent species that emit light characteristic of the heteroatom introduced into the flame. Selection of an suitable filter (394 or 526 nm bandpass) allows selectivity for sulphur or phosphorus, respectively. It is advantageous to use nitrogen as the carrier gas and mix it with oxygen at the column exit. Hydrogen gas is introduced at the burner to initiate combustion.

A high degree of specific molecular identification can be achieved by the gas Chromatograph with various spectroscopic instruments. Although the quantitative information obtained from a chromatogram is usually good, the certainty of identification based only on the retention parameter may be suspect. The combination of chromatographic and spectroscopic techniques provides more information about a Sample than may be obtained from either instrument independently. The chief combined techniques are gas chromatography interfaced with mass spectrometry

(GC-MS), Fourier transform infrared spectrometry (GC-FTIR), and optical emission Spectroscopy (GC-OES).

EXTRACTION, PURIFICATION AND IDENTIFICATION OF SAMPLES OF PESTICIDES

Phosphate substituted organo-pesticides are widely used as selective insecticides in agriculture and consequently can give rise to residues in crops

and food products. The method developed for the analysis of organophosphastes requires partition chromatography, separation and UV measurements. Gas liquid chromatography (GLC) methods for the estimation of organophosphate are also suggested.

High Performance Liquid Chromatography (HPLC) methyl (Figs. 14, 15) has been developed in the laboratory for the analysis of methyl para thion, ciodrin, parathion, dyfonate, EPN, ronnel and trithion. The compounds are separated by using the gradient elution system of mobile phase and the elute monitored by a UV detector at 254 nm. Calculations are based on peak height measurement of the sample and the standard for determining the

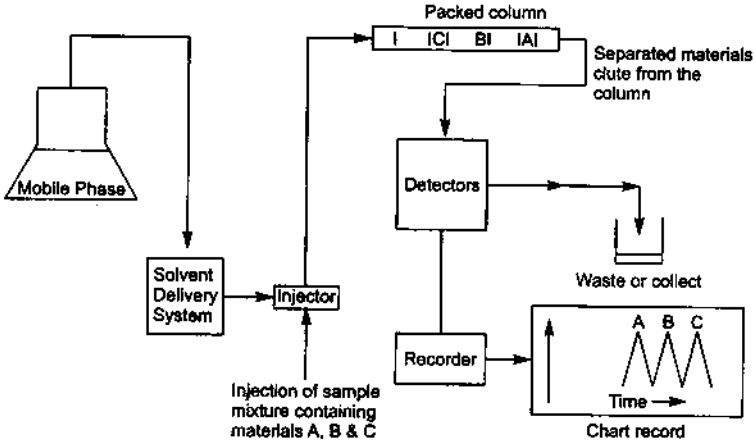
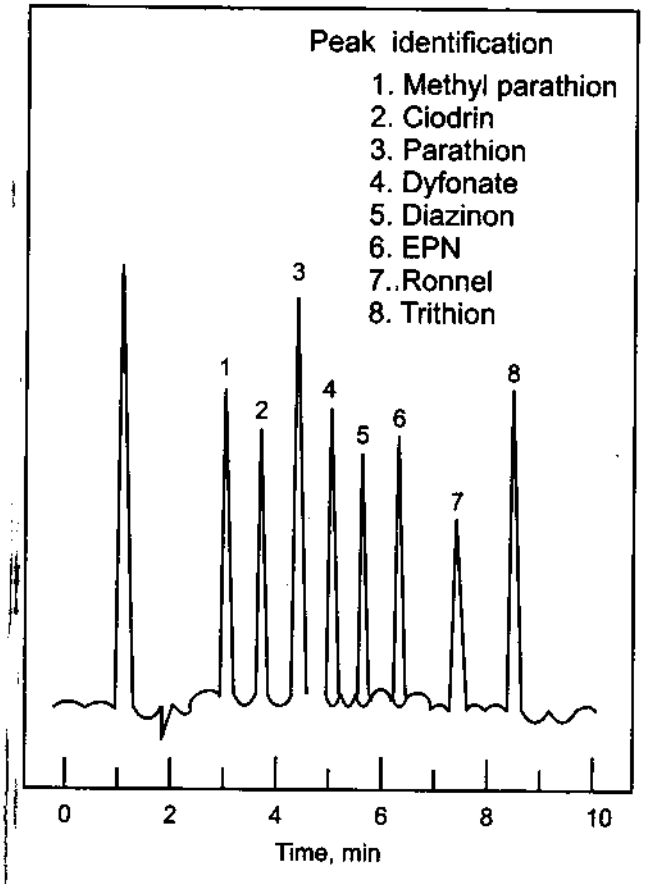


Fig. 14 Block diagram showing the components of an HPLC instrument



Chromatography of Organophosphate insecticides. Column 4.5 × 250 mm packed with 5-μm C_8 bonded-phase particles. Gradient elution: 67% CH_3OH/33% H_2O to 80% CH_3OH/20% H_2O. Flow rate: 2 mL/min. Both used 254-nm UV detectors.

concentration of species. Nuclear magnetic resonance can also be used for detection, analysis and estimation of organophosphate insecticides.

REAGENSTS AND CHEMICALS USED IN ANALYSIS

Commercial grade insecticides etc. are used for chromatographic analysis. The following reagents can be used:

(1) Sulphuric acid, sp. gravity 1.84, (2) Acetone, (3) Hexane, (4) Dichloromethane, (5) Methyl alcohol as eluting solvent (prepared by mixing 67 mL methanol and 33 mL water) (6) Magnesia, prepared as follows: slurry about 500 gm of magnesium oxide with water, heat on steam bath for 30 minutes and filter with suction. Dry overnight at 115 - 130 ⊃ C and pulverise to pass No. 60 sieve. Store in closed container.

EXPERIMENTAL TECHNIQUE : EXTRACTION AND PURIFICATION

Sample (1 litre) is transferred into a two litre separatory funnel and acidified to pH 2 with sulphuric acid. pH is checked with an indicator paper. Add 60 ml of acetone and shake for 2 minutes. Now sample is extracted with 60 mL dichloro methane and hexane (1 : 1), by shaking for 2 minutes and kept for sometime. Water layer is collected in the original sample container and organic phase is collected in a 500 mL Kudema-Danish flask. The extraction is then repeated twice with 50 mL each of dichloromethane and hexane, and the solvent is treated as above. Solvent extract volume is then reduced to 0.5 mL to 0.6 mL under diminished pressure. Few microlitre of this concentrate is then injected to HPLC for analysis of organophosphates In this way dectection of organophosphates in food products is characterized.

The standard conditions for HPLC of organophosphates are as follows:

(1) The column of the HPLC packed with 5 μm C8 bonded-phase particles.

(2) The width and height dimensions of column are 4.5 × 250 mm.

(3) The eluting solvent in this column is used by 67% CH_3OH and 33% water. (80% CH_3OH and 20% water can also be taken as eluant.)

(4) The flow rate of eluting solvent is maintained at 2 mL/minute.

(5) A detector is also attached in this instrument. It is ultra violet detector, which is adjusted at 254 nm for the detection of organo phosphates in food sample.

(6) In this manner, a chromatogram showing the peaks of organophosphates in food sample can be obtained. (Fig. 15)

THIN LAYER CHROMATOGRAPHY FOR CHLORINATED PESTICIDES IN FOOD PRODUCTS

Chlorine substituted pesticides are widely used in agriculture and give rise to their residues to food products and crops. These chlorinated pesticides are mainly benzene hexa chloride (BHC), aldrin, dieldrin, heptachlor, p, p'–D.D.T., p, p'–DDE and p, p'–DDE. These pesticides can be analysed in the

laboratory by TLC method followed by ultra violet measurement. Gas Liquid Chromatography (G.L.C.) methods for the estimation of chlorinated pesticides are also suggested.

Reagents and Chemicals Required

Commercial grade pesticides are used for chromatographic analysis. The following reagents and chemicals can be used:

(1) Methyl alcohol 80 mL + 20 mL water as eluting solvent, (2) Chromogenic reagent: 0.2% $AgNO_3$, (3) Adsorbent, charcoal or aluminium oxide, (4) Silica gel; chromatographic grade, (5) Sulphuric acid of sp. gravity 1.84

Experimental Techniques

One litre of sample is transferred into a 2 litre separatory funnel and acidified with sulphuric acid to pH 2.60 mL of acetone, 30 mL dichloromethane and 30 mL of hexane is added by shaking for 2–5 minutes and organic layer is collected in a 500 mL Kudema–Danish flask. Water layer is collected in original sample container. The extraction is then repeated twice as above. Solvent extract volume is then reduced to 0.5 mL under reduced pressure. This concentrate is taken for thin layer Chromatography analysis.

Prepare the glass plates by coating silica gel slurry of about 0.1 mm thickness. Concentrated sample is applied with the help of capillary tube. Run the plates in methyl alcohol-water (2 : 1) solvent for about 4–5 hours. The chromatogram is developed with chromogenic reagent, 0.2% silver nitrate by keeping the plates at 105°C in air oven.

GAS CHROMATOGRAPHY OF ORGANOPHOSPHATES

To analyse organophosphates of food products by gas chromatography, first of all sample has to be extracted and purified. The chemicals used for this purpose are given below:

Chemicals and Reagents required for extraction and purification

1. Commercial grade insecticides are used as standard.
2. H_2SO_4 of specific gravity 1.84
3. CH_2Cl_2 (dichloro methane)
4. Acetone
5. Hexane
6. pH indicator paper

Apparatus Required

1. Separatory funnel : 2 litre capacity
2. Kudema-Danish flask 500 mL capacity
3. Reduced pressure pump
4. Hot plate

Procedure

1. One litre sample is transferred into a two litre separatory funnel and it is acidified to pH 2 with sulphuric acid.

2. pH is checked with the help of pH indicator paper.

3. Add 60 mL of acetone and shake it for 2 minutes.

4. Now the sample is extracted with 60 mL dichloromethane and hexane in 1 : 1 ratio by shaking for 2 minutes.

5. Keep it for sometime.

6. Water layer is collected in the original sample container.

7. The organic phase is collected in a 500 mL Kudema-Danish flask.

8. The extraction is then repeated twice with 50 mL each of dicloromethane and hexane, and the solvent is treated as above.

9. The solvent extract volume is then reduced to 0.5 mL under reduced pressure.

10. Few micro-litres of this concentrated extract in then used for the analysis of organophosphates by gas-chromatography.

The standard conditions for gas chromatography of organophosphates are as follows :

1. The column of the gas chromatograph is packed with 3% or 101 on chromosorb ω–HP (100–120). (Fig. 16). Its outer wall is made of stainless steel with a height of 6 ft and width of 1/8 inch.

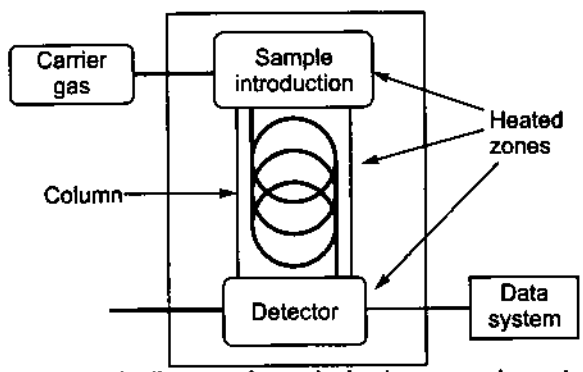


Fig. 16. Schematic diagram of a packed column gas chromatograph

2. The temperature of the gas chromatograph is kept from 170°C for 26 minutes to 200°C for 10 minutes and the flow rate temperature is main- tained at 15°C per minute.

3. The temperature of the Injector and Detector is maintained at 225°C.

4. Nitrogen is used as carrier gas and its flow-rate is maintained at 25 mL/minute.

In the above manner, a chromatogram showing the peaks of organophosphates in the food sample can be obtained.

QUESTIONS

1. What do you understand by the terms food adulteration and contamination ? Give some common examples.
2. Why is food analysis important ? Discuss the safety of food.
3. What are the food standards? Describe the Govt. regulations and recommendations regarding food products.
4. What do you know about the moisture content of food ? How will you determine moisture in vegetable oils and spices?
5. Describe the moisture content of butter, ghee, oil and honey.
6. How will you analyse total ash, water-insoluble ash and acid-insoluble ash in a food sample ?
7. Describe the method to analyse crude fibres in food products.
8. How will you determine the percentage of fat in butter ?
9. What do you understand by crude protein ? Describe the analysis of casein in butter and ghee sample.
10. Give the method of analysis :
    (a) Total reducing sugar in the food containing carbohydrates.
    (b) Reducing sugar after inversion in food having carbohydrates.
    (c) Glucose and fructose ratio in honey sample.
    (d) Commercial invert sugar in honey.
    (e) Starch content in flour sample.
11. Define Fehling's solution A and Fehling's solution B. What is its importance in analysing a carbohydrate sample ?
12. Give the procedure to analyse calcium and sodium in detail in a food sample.
13. Describe the percent calcium as CaO in spice sample.
14. What do you understand by organophosphate containing insecticides ? Give some common examples of it.
15. How will you determine organophosphate insecticide in a food material ?
16. Can you separate, detect and estimate organophosphate insecticides in a food product ?
17. Give the procedure to determine organophosphate insecticides adulteration in food product by HPLC.
18. Give the separation and estimation of organophosphates by Gas Chromatography.
19. Describe the experimental techniques to analyse organophosphates by HPLC.
20. How will you determine chlorinated pesticides in food products by thin layer chromatography method.
21. What are the chlorinated pesticides ? Give some examples. How are they different from organophosphate insecticides ?
22. Describe the analysis of pesticides in food products.

23. Give the extraction and purification of pesticides in food sample.
24. Decribing the method of extraction and purification, how will you determine organophosphates by gas chromatography ?
25. What are the common food adulterants ? How will you analyse crude fibers as adulterant in food product.

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CHAPTER

4

ANALYSIS OF COSMETICS

INTRODUCTION

In cosmetics the conditions which causes body odor are cured by two methods, i.e., deodorants and antiperspirants. The principle of which are antiperspirants that act by contracting the sweat pores by strong astringent action so that perspiration is not freely exuded. Cosmetic deodorants are preparations which mask, remove or decrease perspiration odors, prevent their development or do all these functions. The increasing use of cosmetics and toiletries by teenagers has also created a new and rapidly growing market. This growth was largely due to the development of aerosol deodorant sprays and the subsequent development of aerosol antiperspirants. The tremendous increase in the sale of these products and the variety of products in which deodorants are available indicates the consciousness on the part of both men and women of the offensiveness of malodorous perspiration.

ANTIPERSPIRANTS

A variety of substances which have astringent action inhibit the flow of perspiration. Perspiration is a phenomenon for regulation of body temperature to protect the skin from dryness. Perspiration also eliminates lactic acid which is formed during muscular exercise. Perspiration takes place due to mainly two types of sweat glands i.e. eccrine glands (occurs over almost all body surface) and apocrine glands (occurs in areas like axillar, pelvic region, and around nipples). The secretions of eccrine glands are highly dilute aqueous solution whereas secretion by apocrine glands is oily sticky material.

Common ways to control or reduce odor are :

(i) reduction in apocrine sweating

(ii) removal of secretions of both type of sweat glands as quickly as possible (which is practically not possible)

(iii) checking bacterial growth

(iv) absorption of body odors

Raw Materials : The antiperspirant ingredients include :

(i) Metallic salts commonly that of aluminium and zirconium salts such as aluminium chlorhydrate, aluminium chloride, aluminium sulfate, aluminium zirconium chlorhydrates, etc.

(ii) Antibacterial which are used in cosmetic/toiletries for inhibiting the growth of bacteria includes benzethonium chloride, chlorhexidine acetate, hexane acetate, triclosan, etc.

(iii) Ethyl alcohol which is used as vehicle in deodorant products, is also an antibacterial agent.

Antiperspirants can be formulated in the form of liquid, cream, lotion, stick and powder. Earlier, hexachlorophene was used in deodorants including soaps. Most frequently used antimicrobial in soaps include trichlorocarbanilide (TCC), triclosan etc. in presence of soap, all the antimicrobials (except triclosan) are active against gram positive organisms. Triclosan is active against gram positive and gram negative organisms.

***Formula 1*- Liquid Antiperspirant**

Ingredients		% w/w
Aluminium chlorhydrate (50%)		25.0
Glycerine		5.0
Alcohol		45.0
Borax		2.0
Water	to	100.0
Perfume		qs

Liquid antiperspirants are generally applied as sprays. These are aqueous or hydro-alcoholic solutions of astringent salts with humectants. Small amounts of Tween-40 will diverse perfume. Alcohol present in astringent tends to prevent their hydrolysis in solution and also helps in evaporation. Quaternary ammonium salts such as cetyl pyridinium chloride, cetyl trimethyl ammonium bromide or chlorhexidine can also be used as deodorants in antiperspirants.

***Formula 2* - Liquid Antiperspirant**

Ingredients		% w/w
Aluminium chlorhydrate (50%)		35.0
Cetyl pyridinium chloride		0.5
Propylene glycol		5.0
Alcohol		40.0
Water	to	100.0
Perfume		qs

Antiperspirant creams having the widest consumer acceptance are of vanishing-cream type. Since antiperspirants are acidic in reaction, such perfumes should be selected which are acid stable. The acid-stable emulsifier, compatible with antiperspirants should be used. One such example is the acid-stabilized glyceryl monostearate which can be either used alone or along with other emulsifiers. The anionic emulsifiers used includes sodium lauryl sulfate (SLS), sodium cetyl sulfate and triethanolamine lauryl sulfate. Nonions such as Spans and Tweens can also be used. Fatty materials such as glycerol or glycol esters of fatty acids, cetyl alcohol, white petroleum jelly or mineral oil can be used in small amounts. The concentration of emulsifier helps to determine the final consistency of the cream. Creams which are made with cationic or nonionic emulsifiers generally have a softer consistency than those with an anionic emulsifier. A nonionic emulsifier can be combined with either a cationic or an anionic to form a cream of the desired consistency and texture.

Humectants like glycerine, propylene glycol, polyethylene glycol 400, and sorbitol are used in concentrations of 3-10%. Titanium dioxide is used as opacifier in concentrations of less than 1%. A fabric damage inhibitor like urea is generally used in concentration of 5-10% in case of aluminium salts so as to protect the fabric. Glycine in concentrations of 3 to 10% gives satisfactory fabric protection and is more stable than urea.

***Formula 3* - Cream Antiperspirant**

Ingredients		**% w/w**
Glyceryl monostearate (acid stabilized)		15.0
Spermaceti		5.0
Sodium lauryl sulfate		1.5
Propylene glycol		6.0
Aluminium sulfate		7.5
Urea		5.0
Titanium dioxide		0.5
Water	to	100.0
Perfume and preservativeqs		

Procedure

(i) Heat together the waxes (glyceryl monostearate and spermaceti) to a temperature of 75 to 80°C.

(ii) Heat the water phase (sodium lauryl sulfate, propylene glycol and water) in another container to the same temperature. Add water phase to the wax phase.

(iii) Maintain the temperature for 10-20 min and continue agitation until the emulsion is formed. Cool the emulsion with continuous stirring. Form slurry of the titanium dioxide and incorporate in emulsion.

(iv) Continue to agitate, and cool to 35 to 40°C. Add the aluminium sulfate slowly with agitation and cooling. (Note: the temperature of the cream will rise as the salt dissolves; this rise should be kept at a minimum because too rapid incorporation of the salt causes the emulsion to break or become too soft.) Agitate until the cream is not becomes uniform and the salt dissolved.

(v) Add urea at 40°C or lower, after incorporation of the aluminium sulfate. The urea should be powdered and added slowly with continuous agitation until it is completely dissolved.

(vi) Finally, add the perfume slowly, and continue to agitate until it is uniformly distributed.

Antiperspirant lotions still maintain a reasonable share of the antiperspirant market. For an emulsion type of lotion, the manufacturing procedure is basically the same as that used for a cream. The incorporation of astringent salts in a lotion must be carefully controlled to avoid breaking the emulsion. The finished lotion requires careful testing for emulsion stability with age and change in temperature. The lotion should also be tested for change in viscosity with age.

The lotion antiperspirant are not very popular in the market and whenever prepared should be tested for stability.

***Formula 4* - Lotion Antiperspirant**

Ingredients	% w/w
Glyceryl monostearate (acid-stable)	15.0
Mineral oil	5.0
Petroleum jelly	2.5
Spermaceti	5.0
Glycerine	6.0
Aluminium chlorhydrate (50%)	40.0
Water to	100.0
Perfume and preservative	qs

The antiperspirant sticks usually consist of wax-like matrix which acts as carrier for aluminium chlorhydrate and volatile silicone. Stearyl alcohol is preferred over stearic acid because of its lower melting point.

***Formula 5* - Antiperspirant Stick**

Ingredients	**% w/w**
Aluminium chlorhydrate	18.0
Volatile silicone	45.0
Stearyl alcohol	25.0
Polyethylene glycol distearate 6000	5.0
Carbowax PEG 1540	4.0

***Formula 6* – Antiperspirant Stick**

Ingredients	**% w/w**
Sodium aluminium chlorhydroxy lactate, 10% solution	50.0
Ethyl alcohol	12.0
Propylene glycol	3.0
Sodium stearate	6.0
Perfume	qs

Procedure

(i) The aqueous aluminium salt solution is heated to 60 to 65°C and the alcohol and propylene glycol slowly added with agitation, heating to maintain the temperature at 60 to 65°C.

(ii) The sodium stearate is added with stirring until the soap dissolves. The perfume is finally added, mixed thoroughly, and the solution poured into moulds.

***Formula 7* – Antiperspirant Stick**

Ingredients	**% w/w**
Sodium aluminium chlorhydroxy lactate, 10% solution	50.0
Ethyl alcohol	12.0

Propylene glycol	3.0
Sodium hydroxide	0.75
Stearic acid	5.25
Water	2.0
Perfume	qs

Procedure

(i) Dissolve the stearic acid in warm alcohol. In another container dissolve the sodium hydroxide in the water and combine this with the aluminium complex solution.

(ii) The combined aqueous solution is heated to 65 to 70°C. The alcohol solution is heated in a separate container to the same temperature and added to the aqueous solution with stirring. The soap forms rapidly.

(iii) When the reaction gets complete the mixture is cooled slightly and perfumes are mixed. The product is then poured into moulds.

The antiperspirant powders are the least effective among all other antiperspirant formulations. These are prepared by mixing antiperspirant salt with talcum powder. The powders can also be pressed into powder cakes using binders.

***Formula 8* - Antiperspirant Powder**

Ingredients		**% w/w**
Buffered aluminium sulfate		8.0
Sodium aluminium lactate		8.0
Talcum powder	to	100.0
Perfume		qs

DEODORANTS

Introduction : Deodorants are considered as cosmetics which are used to remove the offensive odor of sweat which is deposited on the skin under warm conditions and undergoes decomposition and chemical changes. This may become offensive even in spite of bathing and hygiene. The ancient people also use perfumes (especially sandalwood and musk) to conceal body odor. Perfumes alone cannot be said to be effective because the odoriferous products of perspiration are extremely difficult to conceal permanently unless a large amount of perfume is used which generally becomes objectionable. There are two types of deodorants; one that intended merely to deodorize perspiration without restricting its flow and the other which deodorize and prevent decomposition through bacteria inhibiting action. Axially odor is produced largely by bacterial action on apocrine secretion. Any compound having antibacterial action can be used. Common antibacterial agents used in cosmetics and toiletries include :

(i) zinc compounds such as zinc phenosulfonate, zinc oxide, zinc peroxide

(ii) organic acids such as benzoic acid, salicylic acid, etc.

(iii) quaternary ammonium compounds such as cetrimide, benzethonium chloride, etc.

(iv) cationic compounds eg. Chlorhexidine acetate

(v) biphenols eg. Hexachlorophene

(vi) phenols eg. Parachlorometaxylenol, dichlorometaxylenol

These can be incorporated as lotions, creams, powders, sticks and soaps.

The deodorant liquids are prepared by dissolving quaternaries in water or 50% denatured spirit. The water-soluble derivatives of chlorophyll and chlorophyllins such as potassium, copper, sodium and magnesium chlorophyllins also possess deodorant properties are used in 0.05% concentrations.

As there is no chemical that possess all the properties for an effective deodorant alone, it is customary to utilize two or more chemicals to produce a product that has the requisite properties. A deodorant may be antiseptic or astringent or both.

***Formula 9* - Deodorant Liquid**

Ingredients	**% w/w**
Chlorhexidine diacetate	0.5
Propylene glycol	2.0
Denatured spirit (50%)	100.0
Perfume	qs

A potassium stearate-stearic acid vanishing cream base can be used for such creams. If chlorinated phenols are used in such preparations, non-ionic emulsifiers should be avoided as these inhibit bacteriostatic properties of these products.

***Formula 10* - Deodorant Liquid**

Ingredients	**% w/w**
Alum	10.0
Aluminium chloride	10.0
Water	75.5
Glycerin	4.0
Perfume	0.5

***Formula 11* - Deodorant Liquid**

Ingredients	**% w/w**
Aluminium chloride	15.0
Water	81.4
Glycerin	3.0
Propyl parahydroxy benzoate	0.1
Perfume	0.5

Procedure

(i) Dry materials are dissolved in water.

(ii) Perfume and glycerin are mixed in thoroughly.

(iii) To facilitate the dispersion of perfume it may be dissolved in a small quantity of alcohol or rubbed up with some talc, which is further filtered out.

***Formula 12* - Deodorant Liquid**

Ingredients	% w/w
Oxyquinoline sulfate	2.0
Aluminium chloride	14.0
Magnesium sulfate	7.0
Water	73.5
Alcohol	3.0
Perfume	0.5

Oxyquinoline sulfate is an excellent ingredient because it is not only a powerful antiseptic but is also an excellent deodorant besides being non-toxic and non-irritating.

***Formula 13* - Deodorant Cream**

Ingredients	% w/w
Glyceryl monostearate	10.0
Stearic acid	5.0
Cetyl alcohol	3.0
Isopropyl myristate	2.0
Potassium hydroxide	0.8
Propylene glycol	10.0
Triclosan	0.2
Water to	100.0
Perfume and preservative	qs

***Formula 14* - Deodorant Cream**

Ingredients	% w/w
Glyceryl monostearate	20.0
Glycerin	10.0
Methenamine	3.0
Water	67.0

***Formula 15* - Deodorant Cream**

Ingredients	% w/w
Glyceryl monostearate	15.0
Titanium oxide	1.0
Petrolatum	3.0
Beeswax	2.0
Formaldehyde (40% sol.)	1.0
Water	78.0

Procedure

(i) All the ingredients except formaldehyde are heated together until a smooth white cream is formed. They are constantly mixed together.

(ii) When the mass cools to 45°C, formaldehyde is stirred in it. This makes the cream greaseless.

The deodorant sticks can be prepared with sodium stearate, humectant, alcohol and perfume resulting in gel formation. They are prepared by dissolving soap in warm alcohol or by saponification of alcoholic solution of stearic acid with sodium hydroxide and adding humectant, deodorant and perfume into it. Finally the solution is poured into moulds to obtain sticks of soap gels. Chlorhexidine diacetate or biothional are effective non-irritant bactericide which can be used in stearate soaps. High quality grade sodium stearate is used whenever clear transparent product is desired. Proportion of stearate varies from 5 to 8.5.

Formula 16 **- Deodorant Stick**

Ingredients	**% w/w**
Sodium stearate	8.0
Sorbitol (solution)	5.0
Isopropyl myristate	3.0
Triclosan	0.2
Alcohol to	100.0
Perfume	qs

The deodorant powder requires dispersion of deodorant in powder which is achieved by mixing and grinding. Dry ingredients should be mixed in suitable blender. The active ingredients should be dissolved in suitable solvent and should be distributed through powder mixture. Perfume may be blended with talc first and then may be incorporated into powder.

Formula 17 **- Deodorant Powder**

Ingredients	**% w/w**
Talc	67.0
Light precipitated chalk	12.0
Boric acid	5.0
Zinc oxide	5.0
Zinc phenol sulfonate	1.0
Perfume	qs

Formula 18 **- Deodorant Powder**

Ingredients	**% w/w**
Purified zinc peroxide	20.0
Talc	39.5
Boric acid	30.0
Zinc stearate	10.0
Perfume	0.5

Formula 19 **- Deodorant Powder**

Ingredients	% w/w
Talc	45.0
Boric acid	30.0
Zinc peroxide	10.0
Zinc stearate	15.0
Perfume	qs

Formula 20 - Deodorant Powder

Ingredients	% w/w
Talc	48.0
Salicylic acid	2.0
Boric acid	40.0
Zinc stearate	10.0
Perfume	qs

COMMERCIALLY AVAILABLE BRANDS

(i) Olay
(ii) Lakme
(iii) Aveeno
(iv) Ayur
(v) Dove
(vi) Ponds
(vii) Fem
(viii) Rexona
(ix) Charlie
(x) St. Ives

LOTIONS

INTRODUCTION

In the field of cosmetics, a lotion is a liquid application (mainly for the skin) to produce a beautifying effect. The main characteristics of lotions is their use as an emollient and soothing effect, astringency, skin freshening effect, bleaching and with medicinal properties. Lotions may be classified as liquid creams (lotions containing gums) and thin lotions (made without gums). Gum and similar substances are added to lotions to facilitate body lubrication and to produce emollient effects. Such preparations are more popular as compared to those lotions which consist mainly of alcohol, water and glycerin used to impart property like astringency. The raw materials used in making lotions include water, glycerin (and other humectants), alcohol, gums (and similar thickening agents), astringents, antiseptics, cooling agents and other miscellaneous ingredients. Water, glycerin and other humectants have already been discussed in earlier chapters.

RAW MATERIALS

Alcohol (usually denatured alcohol) is generally used in lotion formulas. In some cases, isopropyl alcohol is used in place of ethyl alcohol. Methanol which was used from many years is not used nowadays in cosmetics owing to its toxicity on absorption by the skin. Gums consist of complex carbohydrates which usually yield sugars upon hydrolysis. The more common gums used in lotions include gum tragacanth, gum karaya, acacia, Tra-gum, and gum benzoin (a resin). Quince seed, psyllium seed, flax seed (linseed) alginates,

Irish moss, agar agar and starch are other substances used in thickening lotions.

Gum tragacanth is a dried mucilaginous exudation of the tree, *Astragalus gummifer*. In contact with water it swells up to form thick mucilage. It is insoluble in alcohol. Chemically, tragacanth consists of bassorin (water soluble gum) arabin and tragacanthin. Karaya gum is lower in cost than tragacanth, however, it resembles tragacanth and swells to form a mucilage with water. It is derived from the *Sterculia* trees which grow in India, Africa and Australia. It consists mainly of bassorin, a carbohydrate and arabin, an amorphous powder soluble in water and insoluble in alcohol. Acacia, often called Gum Arabic, is a true gum and hence completely soluble in water. It is incompatible with alcoholic tinctures, iron salts, borax. It should not be used with gum tragacanth as it makes thinner mucilage. Acacia is not frequently used in lotions nowadays but is more often used as an emulsifying agent for permanent wave solutions. Recently, a gum of Asiatic origin named Tra-gum possesses unusual colloidal properties. It is edible and has more thickening power than other gums. It is neutral in reaction and produces a flexible, transparent film when the gel dries. It possesses the properties of a stabilizer and coagulant for lotions, dental creams, pastes, emulsions and dispersions.

Gum benzoin is a resin obtained from the bark of *Lithocarpus benzoin*. The best variety is obtained from Siam benzoin and Sumatra benzoin. Gum benzoin is used as preservative as it contains benzoic acid, cinnamic acid, essential oils, small quantity of vanillin, in addition to resins. It is used in lotions because it imparts a pleasing odor, produces an emulsion like clouding when added to thicker lotions. It also possesses slight antiseptic, demulcent and emollient properties. Quince seed obtained from the fruit of quince tree, *Pyrus cydonia*, is mostly used in hand lotions. They consist mainly of amygdalin, emulsion, proteins and glycerides. Its water extractive matter possesses a pleasant soothing and skin softening properties. Since quince seed is quite expensive it is often replaced by mucilages made from flaxseed or linseed or psyllium seed.

From the aquatic plants, mucilages may be obtained from alginates, Irish moss and agar agar. The alginates are derived from seaweed. They have skin softening properties and are safe to use on skin and even to use internally. Irish moss, also termed as Carageenan is derived from a seaweed, *Chondrus crispus*, which grows along the sea coasts of Western Europe and New England. Irish moss extract is used as a substitute for gum tragacanth in lotions and is also used as an emulsifying agent in making tooth pastes and for hair setting preparations. Agar agar consists of the jelly-like water extractive matter from certain algae or seaweeds (mainly of the genus Gelidinum). It mainly contains pectin and para-arabin. It is insoluble in cold water but slowly swells and dissolves in hot water. It is used in limited extent in lotions but is more commonly used in jellies and creams. A common disadvantage of using mucilages is that they are highly susceptible to bacterial and fungal action. Therefore, whenever used in lotions, they should be preserved by the addition of a suitable preservative.

An astringent is a substance which has a property to contract tissues and thereby reduce secretions. The common astringent substances used in lotions are aluminium salts, zinc salts, tannin and witch hazel. The common aluminium salts incorporated in lotions and other cosmetics include ammonium alum, potassium alum, sodium sulfate, aluminium acetate, aluminium acetotartarate, aluminium lactate and aluminium betanaphthol disulfonate. These aluminium salts are usually stable in water solutions, but incompatible in the presence of proteins, tannic acid, alkaline salts, lime water, phenol and contain heavy metal salts. Usually not more than 1% of these astringents are used in lotions. Out of all the zinc salts the more commonly used in lotions are zinc chloride, zinc sulfate and zinc sulfocarbolate. Zinc salts are incompatible with alkalies, proteins, and tannic acid. Zinc chloride which is mainly used in mouth washes is deliquescent, hence must be kept in a well-closed container, in a dry place.

Tannic acid (also known as tannin) is incompatible with proteins, chlorides, heavy metal salts, starch, oxidizing substances, and alkaloids. It is mainly used in external medicinal preparations as a styptic, antipruritic, for the treatment of burns and other skin disorders. It hardens the skin when applied repeatedly in concentrated solution. Owing to its properties it is used in lotions as a perspirant deterrent and as scalp wash. Witch hazel is official in N.F. VI. due to its soothing and astringent action. It is widely used in lotions. It consists of a saturated aqueous extract of the dried leaves of *Hamamellis virginiana* L. The official extract contains 14% of alcohol, 8% of tannin, gallic acid, water, volatile oil and a bitter principle. Its pleasant odor, astringent and soothing properties with its miscibility in alcohol, glycerin and water in all proportions makes witch hazel suitable for use in lotions and other cosmetic preparations like creams, clays, depilatories, and scalp preparations.

Other ingredients used in lotions are antiseptics or bacteriostatic agents. The substances possessing bacteriostatic or germicidal properties are phenol, resorcinol, thymol, substituted phenols, mercury compounds, boric acid, hydroxyquinoline sulfate and alcohol. Alcohol is antiseptic only when it is present in 70% concentration or more. In addition to these substances, benzoates and esters of parahydroxybenzoic acid, salicylates, glycerin, formaldehyde, and chlorobutanol are used to preserve lotions against bacterial action and fungal growth.

When a cooling effect is desired, menthol is quite generally used. It possesses counterirritant by stimulating local anaesthetic and antipruritic properties. It is slightly soluble in water but highly soluble in alcohol. Camphor, phenol, resorcinol, thymol, etc. cause menthol to liquefy and are incompatible with it.

The proper clarification and filtration for making of lotions and liquid oral preparations require tonics. The common causes for clouding and sediment formation or precipitation in a liquid preparation may appear to be eliminated at the time when the product is packaged but may reappear months later. To nullify such problems filter aids are used in conjunction with filters. These include materials such as talc, Fuller's earth, magnesia, powdered asbestos, gypsum, Kieselgurh, diatomaceous earth, paper pulp, saw dust, and other

substances chemically inert to the liquid to be clarified. Activated clays and charcoals are also used for decolorizing or bleaching.

HAND LOTIONS

Introduction: Hand lotions are all-purpose lotions. As hands are the part of body which receives the greatest amount of wear and tear it is natural that lotions should be applied to them most frequently. Generally, their use is recommended before dish washing, house cleaning and other chores that tend to roughen the skin of hands. The main function of hand lotions is therefore, soothing and softening of the roughened hands. While liquid creams have the advantage of replenishing the natural oils of the skin, lotions are used as emollients that are not greasy. They are thereby more suitable and acceptable by many persons for frequent use during the daytime. Typical hand lotion formulas are given below with suitable directions for dispensing them.

***Formula 1* – Hand Lotion**

Ingredients	% w/w
Stearic acid	3.15
Glycerin	6.0
Potassium hydroxide	0.15
Alcohol	8.5
Quince seed	2.25
Perfume	0.5
Preservative	0.15

Procedure

(i) Dissolve the potassium hydroxide in one-third quantity of water and add glycerin to it.

(ii) Heat the remaining water to a temperature of 80°C; add the quince seed and soak for 6 hrs and then strain through muslin.

(iii) Melt the stearic acid, dissolve the perfume in the alcohol; add to the hot potassium hydroxide solution the melted stearic acid and boil for a minute.

(iv) Allow the temperature to drop to about 70°C; and then stir it into the quince seed mucilage.

(v) Stir occasionally until cool, and then slowly add the alcohol, preservative and perfume.

Note : Clearer mucilage is obtained if it is made cold by soaking the seeds or the gum, as the case may be, overnight. In this case the preservative should be dissolved in the water first.

***Formula 2* – Hand Lotion**

Ingredients	% w/w
Stearic acid	1.5
Powdered soap (neutral white)	1.0
Alcohol	4.0
Glycerin	5.9
Borax	2.5

Gum karaya	1.5
Water	82.95
Perfume	0.5
Preservative	0.15

Procedure

(i) Mix the gum karaya with alcohol and stir into half the water (warm)

(ii) When dissolved strain through muslin. (Note: The purer grade of gum employed, the quicker will it dissolve and the higher will be its viscosity)

(iii) Heat the remaining water and dissolve in it borax, powdered soap, glycerin and melted stearic acid and agitate until cool.

(iv) Finally add the mucilage, preservative and the perfume.

***Formula 3* – Hand Lotion**

Ingredients	**% w/w**
Gum tragacanth	1.25
Tincture benzoin	0.3
Alcohol	2.6
Glycerin	5.0
Borax	1.25
Water	86.0
Perfume	0.5

Procedure

(i) Make mucilage of the gum tragacanth with half the quantity of water (warm). Make up the tincture of benzoin.

(ii) Both mucilage and tincture must be strained or filtered well.

(iii) Dissolve the borax in the rest of the water (hot).

(iv) Add the mucilage, then the alcohol in which the perfume has been dissolved.

(v) While stirring in well add the tincture. Mix well, strain again and fill when cool.

***Formula 4* – Hand Lotion**

Ingredients	**% w/w**
Flaxseed mucilage (10%)	43.0
Boric acid	0.2
Alcohol	23.3
Glycerin	10.0
Pineapple juice	23.3
Methyl parahydroxybenzoate	0.2

***Formula 5* – Hand Lotion**

Ingredients	% w/w
Irish moss mucilage (3%)	37.0
Boric acid	0.5
Alcohol	15.0

Glycerin	10.0
Tincture benzoin	0.5
Pineapple juice	36.8
Methyl parahydroxybenzoate	0.2

Procedure

(i) The Irish moss mucilage is made by soaking 3% of cleaned Irish moss in 97% by weight of water.

(ii) After soaking overnight drain off the mucilage and add the required quantity to the above formula. The procedure is similar to the preceding formulas.

***Formula 6* – Menthol Lotion**

Ingredients	**% w/w**
Menthol	0.2
Powdered tragacanth	0.5
Alcohol	9.0
Glycerin	4.5
Water	85.8

Procedure :

(i) Dissolve the menthol in the alcohol and to the tragacanth.

(ii) Mix well and add water and glycerin and mix until a smooth mixture results.

***Formula 7* – Tragacanth and Quince Seed Lotion**

Ingredients	**% w/w**
Tragacanth	0.1
Boric acid	0.7
Alcohol	7.0
Glycerin	7.0
Quince seed	1.5
Sodium benzoate	2.0
Water	81.7

Procedure

(i) Make mucilage of the gum tragacanth with half the quantity of water (warm). Add the quince seed and soak for 6 hrs and then strain through muslin.

(ii) Dissolve the boric acid in the rest of the water (hot).

(iii) Add the mucilage, then alcohol in which the perfume has been dissolved and glycerin.

(iv) While stirring in well add sodium benzoate. Mix well, strain again and fill when cool.

***Formula 8* – Witch Hazel Lotion**

Ingredients	**% w/w**
Quince seed	2.0

Boric acid	2.0
Hot water	8.0
Glycerin	16.0
Witch hazel	71.5
Perfume	0.5

Procedure

(i) Add the quince seed and soak for 6 hrs in half the quantity of hot water and then strain through muslin.

(ii) Dissolve the boric acid in the rest of the water (hot).

(iii) Add witch hazel to the mucilage, then alcohol in which the perfume has been dissolved and glycerin.

(iv) Mix well, strain again and fill when cool.

SKIN TONING LOTIONS

Introduction: Skin toning lotions, also termed as skin freshners, are on an increase use. They have weak astringent, stimulating, and antiseptic properties which are generally used to freshen the skin and to remove residual traces of creams. During manufacturing, it is necessary to dissolve the ingredients in the water, alcohol, and other liquids used and then filter. Sometimes, a small quantity of talc, magnesia or other filter aids is added to facilitate the filtering operations and improve the clarity of the product.

Formulas for preparations of this category follow :

***Formula 9* – Skin Toning Lotion**

Ingredients	**% w/w**
Alcohol	30.0
Lactic acid (85%)	2.0
Glycerin	5.0
Water	62.5
Perfume	0.5

Procedure

(i) Mix perfume with alcohol, add rest of ingredients, and finally filter.

***Formula 10* – Skin Toning Lotion**

Ingredients	**% w/w**
Alcohol	30.0
Glacial acetic acid	1.0
Glycerin	5.0
Water	63.5
Perfume	0.5

***Formula 11* – Skin Toning Lotion**

Ingredients	**% w/w**
Alcohol	15.0
Lactic acid	2.0
Glycerin	5.0

Alum	1.0
Formaldehyde	0.1
Distilled Water	76.6
Perfume	0.3

Procedure

(i) Heat one part of the water and dissolve the alum in it.

(ii) Mix the lactic acid with the remaining amount of water; add the formaldehyde mixed with the glycerin, then add the alcohol and the perfume.

(iii) Mix thoroughly for two hours and allow to stand in a covered container for 3 days, with occasional stirring, then filter.

***Formula 12* – Skin Toning Lotion**

Ingredients	% w/w
Boric acid	2.0
Alum	1.0
Camphor	0.02
Menthol	0.14
Formaldehyde	0.1
Ethylene glycol	4.0
Alcohol	10.0
Distilled Water	82.49
Perfume	0.25

Procedure

(i) Rub the camphor and menthol together until they liquefy.

(ii) Heat one part of the water, dissolve the boric acid in it, with another part of water, and dissolve alum in it.

(iii) With the remaining amount of water; add the formaldehyde mixed with the glycol; then add the boric acid solution and mix again.

(iv) Now add the camphor-menthol solution, alcohol and the perfume.

(v) Mix thoroughly, and finally filter it and pack.

***Formula 13* – Skin Toning Lotion**

Ingredients	% w/w
Alcohol	10.0
Triethanolamine	1.0
Menthol	0.05
Propylene glycol	5.0
Distilled Water	83.7
Perfume	0.25

Procedure

(i) Add the triethanolamine into the water; add the propylene glycol and mix again.

(ii) Dissolve the menthol in the alcohol and mix it with the above.

(iii) Now add the perfume which has been mixed with a small quantity of precipitated chalk.

(iv) Mix the above and filter. Here, glycerin has been replaced by propylene glycol.

***Formula 14* – Skin Toning Lotion**

Ingredients	% w/w
Boric acid	1.0
Witch hazel	15.0
Rose water.	15.0
Alcohol	10.0
Orange flower water	59.0

Procedure

(i) Warm the witch hazel and dissolve the boric acid in it.

(ii) Mix the rest of the ingredients with the orange flower water and add the boric acid solution.

(iii) Mix, and filter the above.

***Formula 15* – Skin Toning Lotion**

Ingredients	% w/w
Boric acid	0.5
Tincture of benzoin	1.25
Glycerin	8.3
Perfume	0.2
Alcohol	40.0
Witch hazel	9.75
Orange flower water	40.0

Procedure

(i) Dissolve the tincture of benzoin and perfume in the alcohol.

(ii) Warm the witch hazel and dissolve the boric acid in it.

(iii) Mix the witch hazel and the orange flower water; add glycerin; then add the tincture and alcohol.

(iv) Mix thoroughly, and filter the above.

ASTRINGENT LOTIONS

Introduction: They are intended to remove excessive oiliness and to make coarse pores less noticeable. Oiliness is usually caused by eating a diet containing an excessive amount of greasy or oily foods. Alums, the double salts of aluminium sulfate and alkali sulfates or ammonium sulfate, are potent astringents. Extremely weak solutions of such salts as aluminium sulfate or aluminium chloride will produce a "puckering" when applied to the tip of the tongue. The more concentrated forms are used for styptic purposes, where they function by coagulating the blood protein to form a tough clog. The corrosive and irritant effects of high concentrations of astringent salts are due to the liberation of strong acids. Proper formulation can prevent this undesirable

action. Different methods have been developed for testing the astringent action of a substance

➤The precipitation of protein can be measured

➤The difference in the extensibility of tissues before and after treatment with a solution of the substance

➤The increased resistance of red corpuscles to hemolysis.

➤However, the most reliable method of measuring astringency is by taste.

***Formula 16* – Astringent Lotion**

Ingredients	% w/w
Boric acid	3.0
Alum	1.3
Formaldehyde	0.2
Glycerin	5.0
Alcohol	10.0
Water	80.0
Perfume	0.5

Procedure

(i) Dissolve the boric acid in one part of water with heat and other part with alum.

(ii) Mix the formaldehyde with the glycerin, and the perfume with the alcohol.

(iii) Then add to the rest of the water the alum solution, the boric acid solution, the formaldehyde mixture and the perfume solution.

***Formula 17* – Astringent Lotion**

Ingredients	% w/w
Alum	0.75
Zinc sulfate	0.1
Glycerin	10.0
Alcohol	10.0
Water	78.65
Perfume	0.5

Procedure

(i) Dissolve the alum in one part of the water, and the zinc sulfate in the glycerin.

(ii) Mix the zinc sulfate solution with the remainder of the water; add the alum solution and then the alcohol.

(iii) Allow to stand for 24 hrs and filter.

***Formula 18* – Astringent Lotion**

Ingredients	% w/w
Acetic acid (glacial)	2.0
Alum	1.0
Zinc sulfate	0.1

Glycerin	9.0
Menthol	0.05
Tincture of benzoin	2.0
Alcohol	15.0
Water	70.35
Perfume	0.5

Procedure

(i) Dissolve the alum in one part of the water, and the zinc sulfate and acetic acid in the glycerin.

(ii) Mix the menthol, perfume and tincture of benzoin with the alcohol.

(iii) To the remainder of the water add the acetic acid solution, the alum solution, the zinc sulfate solution and the alcohol mixture.

***Formula 19* – Glycerin Lotion**

Ingredients	% w/w
Aluminium sulfate	1.0
Glycerin	4.5
Triethanolamine	0.5
Alcohol	30.0
Water	63.5
Perfume	0.5

***Formula 20* – Mild Astringent Lotion**

Ingredients	**% w/w**
Borax	0.15
Zinc phenolsulfonate	0.5
Camphor	0.6
Menthol	0.35
Alcohol	30.0
Perfume	0.6
Glycerin	3.0
Water	65.15

MEDICATED LOTIONS

Introduction: Blemishes and disorders detract skin beauty. Many medicinal skin treatment are used for the treatment of skin diseases as acne, eczema, etc. Some formulas of medicated lotions are also used to afford at least temporary relief to the less serious skin disorders.

***Formula 21* – Medicated Lotion**

Ingredients	**% w/w**
Lecithin	1.0
Sodium lauryl sulfate	3.0
Chlorothymol	0.1
Alcohol	22.0
Water	67.9
Chloroform	6.0

Procedure

(i) Dissolve the lecithin in the chloroform, and the chloro-thymol and sodium lauryl sulfate in the alcohol.

(ii) Add water and mix the solutions.

***Formula 22* – Medicated Lotion**

Ingredients	% w/w
Menthol	0.2
Phenol	0.07
Bismuth subnitrate	5.0
Zinc oxide	5.0
Glycerin	10.0
Formaldehyde	0.2
Rose water	79.53

Procedure

(i) Rub menthol and phenol together with a little quantity of glycerin; add the formaldehyde.

(ii) Mix the remainder of the glycerin in rose water; sift in the zinc oxide and bismuth subnitrate.

(iii) Mix thoroughly and add the menthol-phenol solution.

***Formula 23* – Mild Astringent Lotion**

Ingredients	% w/w
Zinc sulfate	2.0
Sulfurated potash	3.0
Colloidal sulfur	3.0
Glycerin	4.0
Distilled water	50.0
Rose water	38.0

Procedure

(i) Dissolve the sulfurated potash in a small quantity of water. Similarly, dissolve zinc sulfate in water.

(ii) Mix sulfur and glycerin and stir into the rest of the water.

(iii) Add rose water, then the solution of zinc sulfate.

(iv) Finally mix in the sulfurated potash solution.

***Formula 24* – Resorcinol Lotion**

Ingredients	% w/w
Resorcinol	3.0
Glycerin	13.0
Alcohol	44.0
Rose water	40.0

***Formula 25* – Alkaline Triethanolamine Lotion**

Ingredients	% w/w
Triethanolamine	2.0
Potassium carbonate	1.0

Witch hazel	67.0
Rose water	30.0

***Formula 23* – Calamine-Oxyquinoline Lotion**

Ingredients	% w/w
Calamine	3.0
Oxyquinoline sulfate	0.8
Zinc oxide	6.0
Glycerin	9.0
Distilled water	81.2

Triethanolamine has been recommended that this agent penetrates and emulsifies the accumulated sebaceous matter, thus facilitating ready removal.

DEPILATORIES

DEPILATORY

Introduction: Different measures have been tried by men and women since antiquity for the removal of undesired hairs in order to improve one's personal appearance. Women, in particular do not wish to have prominent hairs, particularly on face. Also, the spread of contemporary fashion which dictates the use of sleeveless gowns and tops or cosmetic stockings requires the removal of hair under the arms and on the legs. An extensively used classical depilatory *Rhusma turcorum*, containing natural arsenic trisulfide, quicklime, and starch was used in the form of a paste with water by certain African tribes as a hair-removing agent. High androgen level or decrease in estrogen production is prone to cause an excessive hair growth (hirsutism) in women. Occasionally, hirsutism is observed during puberty or in pregnancy but is more common during the menopause.

Hair consists mainly of amino acids, prominently containing sulfur and cystine. Since hairs are not readily dissolved or destroyed they require careful consideration for their removal. The mechanical methods used for the removal of undesired hair are shaving, electrical galvanization and epilation or pulling out the hair. Shaving is usually a semiweekly procedure, which is done by especially designed razors and blades with improved cutting edges and using elegantly formulated shaving creams. However, this process is considered tedious and unpleasant because of cuts, scratches and alleged re-growth of stubbles. Removal of hair by ionizing radiation (e.g., X-ray) is very dangerous as it is capable of permanent destruction of the hair follicle and loss of hair. Therefore, this method should never be employed for the purpose of cosmetic removal of excessive hair. The other electrical methods consist of the use of galvanic current and high frequency current.

Hair can be permanently removed by electrolysis. It involves the introduction of a needle into the hair follicle which results in destruction of the papilla. Earlier electrolysis machines used weak direct current but newer methods involve electrocoagulation with a high frequency alternating current which is faster. These methods are costly, time-consuming and associated with the risk of possible infection and scarring. The overall result is largely dependent on the operator performing the method. Bleaching of the hair on

upper lip and cheeks, results in concealing the unwanted hairs from the face. Rubbing the hairy area with an abrasive (e.g., pumice) may break the hair shaft. This method is inconvenient to use and often too abrasive to the skin. Although use of razors is widespread, another quite generally employed method of removing unwanted hair involves the use of epilating waxes and the application of depilatory creams.

EPILATORY WAXES

Introduction: Epilants or "hair-pull" is semisolid adhesive mixtures. They consist of wax-resin composition containing a mixture of waxes applied in the molten state to the hirsute area and allowed to solidify, so that the hair sticks in the waxy material. By stripping the layer of congealed wax from the skin, the hair is uprooted with the wax film. The second one, adhesive semi-solid compositions are permanently sticky at room-temperature and are applied usually on a flexible backing material, such as fabric. Due to the pain occasions in this stripping process and allergic reactions to the adhesives, these epilants are not often used as chemical depilatories. Epilatory waxes are popular and are comparatively easier to make. Such formulas are given below along with their method of preparation:

***Formula 1* - Epilating Waxes**

Ingredients	% w/w
Rosin	58.0
Yellow beeswax	20.0
Paraffin	16.0
Petrolatum	5.0
Perfume	1.0

***Formula 2* - Epilating Waxes**

Ingredients	% w/w
Rosin	42.0
Beeswax	37.0
Carnuaba wax	6.0
Mineral oil	14.0
Perfume	1.0

***Formula 3* - Epilating Waxes**

Ingredients	% w/w
Rosin	64.0
Beeswax	8.0
Carnuaba wax	24.0
Linseed oil	3.0
Perfume	1.0

Procedure

(i) Melt the rosin and the waxes, mix and add petrolatum (or mimeral oil).

(ii) When the temperature drops Heat to about 60°C; add the perfume.

(iii) Pour the melted mass into suitable moulds or container.

During use, this wax is melted and painted over the surface to be dehaired.

CHEMICAL DEPILATORIES

Introduction: Within the last few decades, cosmetic depilatory creams have become popular. They are used for temporary removal of undesired hairs that projects on the outer surface of the skin. An ideal chemical depilatory formulation should possess the following properties :

(i) It should convert human hair to a soft plastic mass within 2 to m5 min; which can be easily removed from the skin by wiping or rinsing.

(ii) It should be non-toxic systemically and non-irritating to the skin even on prolonged contact.

(iii) It should be easily applied and spreadable on skin.

(iv) It should be economical to use.

(v) It should be stable in the tube or container used.

(vi) It should be cosmetically elegant, odorless or slightly perfumed, white or neutral in color.

(vii) It should not cause stain on the skin or clothing.

Usually, the depilatory should be applied and allowed to remain on the skin for about five minutes (the time being governed by the thickness of the hair). Generally, coarse hair will requires a longer period of time. The depilatory should be washed off with water; skin patted dry and treated with cold cream. In case, if not all the hair is removed following the first application, the used should be warned not to repeat the application for several days.

Raw Materials : Chemical depilatories are available in the form of liquids, pastes and powders. The most popular depilating chemical are the metal sulfides such as sulfides of lithium, sodium, potassium, cesium, magnesium, calcium, strontium, barium, aluminium, arsenic and tin (alkali and alkaline-earthy sulfides). Earlier, barium sulfide was used as a depilatory. However, because of its poisonous nature, it has largely been abandoned. Use of sodium and potassium sulfides is usually confined to liquid depilatories containing glycerine to allay irritation. Pastes containing calcium sulfo-hydrate or strontium sulfide have a strong odor of hydrogen sulfide owing to hydrolysis. This unpleasant odor is controlled by adding alkali to the formulations so that the pH is 11 or above. Moreover, sulfide preparations have the propensity to convert to shades of green, blue, or gray (especially if the pH is lower than 11) owing to oxidation and reaction with trace metals, such as iron.

A problem associated with strontium sulfide preparations is that strontium hydroxide often crystallizes out soon after the product has been manufactured. The best way to formulate depilatories containing metallic sulfides appears to be oil-in-water emulsions compounded with emulsifying agents which are not affected by the high alkalinity and electrolyte concentration.

Most of the sulfide depilatories have negligible odor when freshly made but develop an obnoxious smell on standing. Moreover, a strong odor of

hydrogen sulfide is produced on removing the preparation from the skin with water. This can be avoided by first scrapping off the depilatory with a spatula or wiping it with a cloth or cleansing tissue before washing with water. The unpleasant odor of depilatories when applied to the skin is very difficult to control with an ordinary perfume. The common perfume materials used are aromatic alcohols, ketones, anise, safrol, and rose.

To control the discoloration of sulfide depilatory formulas on aging the addition of non-reactive pigments of great covering power such as X-ray grade barium sulfate, lithopone, zinc oxide, tin oxide, or titanium dioxide to pastes and liquids has been patented. Similarly, calcium carbonate, strontium carbonate, aluminium hydroxide are used as fillers or amphoteric buffers. Lauryl, cetyl and stearyl alcohols are stable at a high pH and serve to add emollient property. The irritating action of alkali sulfides can be reduced by the addition of casein or its salts and by albumin degradation products. Non irritating compositions containing strontium sulfide with the addition of hydroxylquinoline sulfate are available to minimize skin damage. The addition of a quaternary ammonium salt helps to stabilize sodium sulfide depilatories. Examples of depilatory powders are given as follows:

***Formula 1* - Depilatory Powder**

Ingredients	% w/w
Barium sulfide	33.0
Titanium dioxide	18.0
Corn starch	48.5
Menthol	0.25
Perfume	0.25

Procedure

(i) Dissolve menthol in perfume oil; add a little quantity of starch to it

(ii) Sift the titanium dioxide and barium sulfide into mixer

(iii) Mix and sift the remaining quantity of starch

(iv) Mix (iii) in the menthol- perfume mixture

(v) Mix the entire batch for about half an hour and fill in container.

Note : Mix with water at the time of application.

***Formula 2* - Depilatory Powder**

Ingredients	% w/w
Strontium sulfide	35.0
Zinc dioxide	23.0
Corn starch	35.0
Powdered soap	5.0
Benzocaine	0.2
Perfume	1.8

***Formula 3* - Depilatory Powder**

Ingredients	% w/w
Barium sulfide	28.0

Zinc dioxide	25.0
Wheat starch	40.0
Powdered soap	5.0
Benzocaine	0.2
Rose perfume	1.8

Procedure

(i) Mix benzocaine and perfume oil; add a small quantity of starch to it

(ii) Sift the titanium dioxide and barium sulfide with the remaining amount of starch into the mixer and mix

(iii) Add the powdered soap and the perfume mixture

(iv) Close the mixer and mix for about half an hour and fill at once.

Note: Mix with water at the time of application.

***Formula 4* - Depilatory Powder**

Ingredients	**% w/w**
Strontium sulfide	35.0
Corn starch	35.0
Talc	28.0
Menthol	0.2
Perfume	1.8

***Formula 5* - Depilatory Powder**

Ingredients	**% w/w**
Strontium sulfide	25.0
Barium sulfide	10.0
Corn starch	35.0
Calcium carbonate	28.0
Perfume	2.0

***Formula 6* - Depilatory Powder**

Ingredients	**% w/w**
Barium sulfide	15.0
Strontium sulfide	20.0
Talc	14.0
Calcium carbonate	24.0
Corn starch	25.0
Lavender oil	2.0

Depilatory pastes are more difficult to make as powders. The paste, cream, or lotion must be of proper consistency, capable of being localized at the site of application, easily spreadable, and non-drying (i.e. provide wetting action). It should maintain (build-up) around the hair shaft and cling to the hairy area. A cream-like consistency may be achieved by using natural or synthetic thickening agents, such as tragacanth, karaya, guar gum, methyl or hydroxyethyl cellulose, polyvinyl alcohol, and chemically modified starches and sugars or by using emollients such as cetyl or stearyl alcohol. Depilatory

liquids are easy to make. They are mixtures of strontium or sodium sulfide, water and glycerin to which starch, colloidal clay or saponin may be added.

***Formula 7* - Depilatory Paste**

Ingredients	% w/w
Strontium sulfide	30.0
Corn starch	15.0
Precipitated chalk	15.0
Glycerin	10.0
Water	29.0
Perfume	1.0

Procedure

(i) Add glycerin to the water.

(ii) Mix strontium sulfide to it.

(iii) Sift in the precipitated chalk and starch, then add perfume.

(iv) Mix thoroughly until a smooth paste is obtained. The consistency of the paste can be altered by means of the starch and chalk mixture.

***Formula 8* - Depilatory Paste**

Ingredients	% w/w
Strontium sulfide	15.0
Barium sulfide	15.0
Corn starch	20.0
Talc	10.0
Glycerin	18.0
Water	20.0
Perfume	2.0

Procedure

(i) Mix barium and strontium sulfide with glycerin and water.

(ii) Mix the starch and the talc, sift the dry materials into the sulfide solution.

(iii) Mix well and add the perfume.

***Formula 9* - Depilatory Paste**

Ingredients	% w/w
Fresh sulfurated lime	25.0
Colloidal clay	5.0
Corn starch	15.0
Precipitated chalk	35.0
Glycerin	19.0
Perfume	1.0

***Formula 10* - Depilatory Paste**

Ingredients	% w/w
Sodium sulfide	4.0
Calcium hydroxide	4.0

Glycerol	1.0
Kaolin	32.0
Water	59.0

Formula 11 **- Depilatory Paste**

Ingredients	**% w/w**
Barium sulfide	8.0
Calcium carbonate	32.0
Powdered soap	4.0
Glycerol	2.0
Water	54.0

Formula 12 **- Depilatory Paste**

Ingredients	**% w/w**
Strontium sulfide	30.0
Zinc oxide	8.0
Glycerol	8.0
Methyl cellulose	2.5
Menthol	1.0
Water	50.5

Formula 13 **- Depilatory Liquids**

Ingredients	**% w/w**
Sodium sulfide	9.0
Glycerin	10.0
Alcohol	2.0
Water	78.5
Perfume	0.5

Procedure

(i) Dissolve the sodium sulfide in about half the quantity of water.

(ii) Add the glycerin and mix. Add the remaining quantity of the water to it.

(iii) Finally add the perfume, dissolved in the alcohol.

(iv) Mix well and filter.

Formula 14 **- Depilatory containing thioglycolate**

Ingredients	% w/w	% w/w
Calcium thioglycolate	5.4	5.4
Calcium hydroxide	6.8	6.6
Strontium hydroxide	3.4	3.7
Cetyl alcohol	4.3	6.0
Calcium carbonate	22.4	–
Brij 35	1.2	1.0
Perfume	0.3	0.2
Distilled water qs	100.0	100.0

A cream formula containing thioglycolate as an active principle in chemical depilatory tends to lose a part of its depilating potency if exposed to air. Reduction of alkalinity by atmospheric carbon dioxide and oxidation of thiol retards depilation and promotes color formation in the cream. Therefore it is important to protect the formulation from atmospheric degradation. Lotion depilatories are usually packaged in jars whereas creams in collapsible tubes. Air spaces between cap and product surface in the jar between the cream and the crimp seal in the tube should be reduced to a minimum. The high degree of chemical reactivity of the depilatory formula makes it essential that nonreactive plastic seals and caps be used for jars and tubes. Therefore it is better to use wax-lined tin or preferably lead for the bodies of collapsible tubes. The reactivity of thioglycolate towards metal containers has lead to the usage of collapsible plastic containers for packaging (especially those made of polyethylene or polyvinylchloride).

ASSIGNMENTS

1. Differentiate between anti-perspirants and deodorants.
2. What are anti-perspirants? Explain giving two examples.
3. What are deodorants? What are the raw materials used in the manufacturing of deodorants?
4. Explain giving examples of different types of deodorant formulations?
5. What are the various antibacterial agents used in antiperspirants?
6. What are the different raw materials used for the manufacturing of antiperspirants?
7. Explain the answer giving suitable formulas.
8. What are the common ways to reduce or eliminate body odor? Give relevance of the use of antiperspirants as a cosmetic preparation.
9. What are the raw materials used in Lotions?
10. Give a detailed account of common gums used in lotions.
11. What is the use of astringent substances in lotions. Justify the answer giving relevant examples.
12. Give a note on filtration and clarification in the preparation of lotions.
13. What are the different types of lotions used in cosmetics. Give a detailed account of hand lotions.
14. Giving examples, explain the various ingredients used in skin toning lotions.
15. What are the different methods for the removal of unwanted hairs from the body?
16. Define "hair-pulls"? Explain giving a suitable example.
17. What are the ideal properties of a chemical depilatory formulation.
18. Explain giving suitable formulation the different raw materials used for preparing chemical depilatories.

Multiple Choice Questions

1. Oxyquinoline sulfate is used in deodorant because it is
   (a) non-toxic (b) non-irritant
   (c) Both (d) None of these

2. Sorbitol is used in deodorant sticks as a
   (i) Humectant (b) Gelling agent
   (c) Diluent (d) All of these
3. The anionic emulsifier used in antiperspirant cream is
   (a) SLS (b) Tragacanth
   (c) Spans (d) Tweens
4. Urea is used as an ingredient in some antiperspirant creams. It is used as
   (a) Opacifier (b) Humectant
   (c) Fabric damage inhibitor (d) None of these
5. A common opacifier used in antiperspirant formulas is
   (a) Glycine (b) Titanium dioxide
   (c) Spans (d) Urea
6. Triclosan is used as an ingredient of antiperspirant formulations. It is a
   (a) Antibacterial agent (b) Metallic salt
   (c) Vehicle (d) Humectant
7. Gum tragacanth is obtained from the tree of
   (a) *Astragalus gummifer* (b) *Sterculia*
   (c) *Acacia arabica* (d) None of these
8. Benzoin is a
   (a) Gum (b) Resin
   (c) Both (d) None
9. _____ is used as a substitute for gum tragacanth in lotions and as emulsifying agent in making toothpaste and hair setting preparartions.
   (a) Carageenan (b) Agar agar
   (c) Irish moss extract (d) Alginates
10. Quince seed is obtained from the fruit of
    (a) *Psyllium* (b) *Pyrus cydonia*
    (c) Siam benzoin (d) *Sterculia*
11. Gum benzoin can also used as preservative because it contains
    (a) Benzoic acid (b) Cinnamic acid
    (c) Both (d) None of these
12. Zinc chloride is used in lotions as well as in mouth washes. However, preparation containing it must be kept in well-closed containers. This is because zinc chloride is
    (a) Humectant (b) Deliquescent
    (c) Irritant (d) Photosensitive
13. Witch hazel is used in lotions owing to its soothing and astringent action. It is obtained from dried leaves of
    (a) *Hammelis virginiana* (b) *Terminalia chebula*
    (c) *Astragalus gummifer* (d) None

14. Filter aid used in lotions _____
    (a) Talc (b) Fuller's earth
    (c) Powdered asbestos (d) All of these
15. The common humectants used in lotions are
    (a) Glycerin (b) Sorbitol
    (c) Propylene glycol (d) All of these
16. Another name for skin toning lotions is
    (a) Skin freshners (b) Hand lotions
    (c) Astringent lotions (d) None
17. A classical depilatory, containing natural arsenic trisulfide is
    (a) *Rhusma turcorum* (b) *Pyrus cydonia*
    (c) *Astragalus gummifer* (d) None
18. Hair consists of amino acids, mainly
    (a) Sulfur (b) Cystine
    (c) Both (d) None of these
19. The permanent removal of unwanted hairs can be achieved by
    (a) *X*-rays (b) Electrolysis
    (c) Shaving (d) Galvanization
20. The other name for "hair-pull" mixtures is
    (a) Epilant (b) Depilatory
    (c) Both (d) None of these
21. Barium sulfide was earlier used as depilatory. Its use has been abandoned nowadays because it is
    (a) Poisonous (b) Irritant
    (c) Unpleasant odor (d) Stained fabric
22. Common perfume materials used in depilatories is
    (a) Aromatic alcohol (b) Anise
    (c) Safrol (d) All of these
23. Thickening agents used in preparing depilatory pastes includes
    (a) Tragacanth (b) Karaya
    (c) Methyl cellulose (d) All of these
24. Because of the reactivity of thioglycolate with metal containers, the plastic container should be used, containing
    (a) Polyethylene (b) Polyvinyl chloride
    (c) Both (d) None of these

ANSWERS

1. (c), 2. (a), 3. (a), 4. (c), 5. (b), 6. (a), 7. (a), 8. (b), 9. (c), 10. (b), 11. (c), 12. (b), 13. (a), 14. (d), 15. (d), 16. (a), 17. (a), 18. (c), 19. (b), 20. (a), 21. (a), 22. (d), 23. (d), 24. (c).