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Practical of General Chemistry

for the First Years of Faculties of Sciences and Medicine

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First edition 2023

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College of Education – University of Kordofan

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Introduction of Book

We are pleased to dedicate to you our humble effort after God's grace to accomplish it in this way. We hope to God that every reader and every diligent, educated person interested in the science of chemistry will find what he or she is looking for in this simple work. This work has been accomplished to enrich libraries with a specialized reference for practical experiments in general chemistry in English for first-year university students in chemistry and medicine to serve as a guide to improve the development of their cognitive abilities. This book is nothing but a new addition to a series of valuable books that we will present in successive editions. An elite group of scientific cadres in the specialty of chemistry participated in refereeing this book.

In this book, we aim to simplify the laboratory experiments for our students in many scientific departments, in the science, medical science, and engineering sectors, pharmaceutical, agriculture, by introducing them to the basics of chemistry. Furthermore, learn the basic practical concepts in chemistry and understand how to apply these concepts to their lives and the world around them in a simplified scientific manner. In this regard, the basic information and principles from laboratory chemistry experiments for first-year university students majoring in chemistry and medicine were selected, arranged, and included in this book, divided into five chapters:

The first chapter includes the methods of performing analytical reactions, conditions of the analytical reactions, Personal and general laboratory safety, and methods of preparing the laboratory report. The second chapter discusses systematic identification of acidic radical in inorganic Salts, investigation of unknown anions in inorganic Salts group HCl acid reagent, investigation of Conc H_2SO_4 Group anions, and investigation of qualitative analysis of barium chloride group (Phosphate ions and Sulphate ions). It is also discussed investigation of qualitative analysis of cations, analysis of group I group II group III group IV group V cations, and group VI cations, and general characteristic of cations. The third chapter it contains volumetric analysis, standard solution, Titration elements, titration and acid-base neutralization, redox titrations, and iodometric titrations. Chapter four discussed qualitative analysis of organic compounds includes, elemental analysis in organic compounds, detection of elements others than carbon and hydrogen in organic compounds, qualitative organic functional groups tests, reaction with acidified potassium dichromate solution, test for carbonyl compounds group, test for carboxyl group, detection of unsaturated fatty acids, esterification test. Chapter five discussed, biochemistry experiments involved homo-polysaccharides and hetero-polysaccharides, glycerol, action of acids on carbohydrate, reducing sugars and non-reducing sugars, phenyl hydrazine test, determine saponification value, determine iodine value, tests based on precipitation reactions of proteins, analysis of urea and uric acid, analysis of milk, determination of titratable acidity and ammonia in urine,

Finally, at the end of the book there are methods for preparing reagents used to conduct experiments. In order to know what is up-to-date in this specialty, we have been keen to provide the student with many scientific and specialized references. We ask God that this book will benefit every student of knowledge in the field of pure and applied chemistry, the medical field, and other fields concerned with this type of science.

The authors

Content

Chapter

1

Analytical Chemistry

Chapter One

Analytical Chemistry

1. Introduction to Analytical chemistry

This book is mainly intended for students studying applied sciences. It is part of the course material for students attending the general and basic chemistry. As it covers a broad range of subjects on the basic as well as the practical aspects of general chemistry, it is likely that it will be also useful for any student attending different theoretical or practical and general chemistry biochemistry courses.

It laid strong background and experience in chemical study and help to successful completion of the course entitled “general and biochemistry”, which is taught as part of the general chemistry B.Sc. program. The book where the practical principles are also discussed interactively with students and the classical practical where the same is accomplished by performing experiments and analyzing experimental data in a first-hand manner. We have put special emphasis on presenting demonstrations sets that will make the students face “real-life” laboratory situations. with students working in groups on finding the solution and the teacher being involved only as a discussion moderator. The experience of the authors help gathered during more than ten years of practice.

This book mainly deals with techniques used to study qualitative and quantitative analysis and the methods on carbohydrates, lipids and proteins are discussed as parts of organic chemistry course. By completing this book students will acquire practical knowledge regarding the techniques used to investigate the properties of macromolecules. Students will become familiar with commonly used lab ware and instrumentation of a general and biochemical laboratory, learn the requirements for sterile work, and will be able to predict their results properly. They learn how to correctly use chemical units of measure and to make solutions for basic chemical experiments. They will be able to determine the acids, bases and macromolecules by taking into account their chemical environment. They will be able to design protocols for the preparation methods of analyzing matters. They will become familiar with the physicochemical background of inorganic and organic salts. The authors wish the students and all readers an enjoyable experience in entering the field of chemical and laboratory. The collection information's of this book was organizing including the general idea, theory, apparatus, chemical materials requirement of any experiment and describing the scientific methods which used including all steps details. This book will contribute for understanding the principal and develop the student practical skills and knowledge for applied in the scientific methodology.

Many different definitions exist for' chemical analysis" but it may be reasonably stated as the application of a process or series of processes in order to identify and/or quantify a substance, the component of a solution or mixture, or determination of the structures of chemical compounds. This means the scope of analytical chemistry is very broad and embraces a wide range of manual, chemical and instrumental techniques and procedures. Analysis method is one of the fundamental chemical subjects, which is the basis for the subsequent study of pharmaceutical chemistry, forensic chemistry, technology of drugs, etc. It consists of two main parts: qualitative and quantitative analysis. The aim of analysis teaching is creating the theoretical basis of chemistry, as well as providing practical skills and experience of making it to the students.

1.1 The methods of performing analytical reactions

Analytical reactions are performed by the dry or wet method. In the first case, the sample tested and the analytical reagents are taken in the solid state, and they are subjected to heating at high temperature. They are:

1. Flame test – the test involves introducing a sample of the element or compound to hot, non-luminous flame, and observing the color that appears in result;

2. Bead test – the reactions for borax bead formation – sodium tetraborate $Na_2B_4O_7 \cdot 10H_2O$ when heating combines readily with a number of colored transition metal oxides, such as Co, Ni, Cr, Cu, Mn, etc., to form the corresponding metaborates possessing characteristic colors;

3. The reactions of caking with some dry reagents (Na_2CO_3, $KClO_3$, KNO_3) for obtaining the characteristically colored products. The reactions performed by the dry method are usually used in the preliminary tests.

4. The reactions performed by the wet method are the main ones in qualitative analysis.

5. They are accompanied by an external effect: changing of the solution coloration, forming or dissolving the precipitation, evolving of a gas.

1.2 Conditions of the analytical reactions

While performing the analytical reaction in qualitative analysis the following requirements should be taken in consideration: Creation and maintenance of a certain value of the solution pH in the reaction; and certain concentration of the reactants. Requirements to the analytical reactions is their sensitivity:

1. The sensitivity of a reaction is the least concentration of ions, which can be found with the help of this reaction in the definite conditions of its performance.

2. The sensitivity of the analytical reaction depends on the conditions of its performance: pH medium, solution ion strength, the presence of side-reactions, etc.

3. To increase the sensitivity of reactions the concentration of the substance in the solution is increased, more often by evaporation.

4. The analytical reactions used in qualitative analysis are divided into specific and selective ones.

5. The specificity characterizes the ability to detect the presence of an unknown element in the presence of other elements.
6. Specific reactions give an analytical effect only with one individual substance.
7. The systematic course of analysis is that the complex mixture of ions is separated by the so-called group reactants into some separate groups.

Objective: is to determine the cation and ions present in a given salt. Qualitative analysis is a method of Analytical chemistry that deals with the determination of elemental composition of inorganic salts. The common method for testing any unknown sample is to make its solution and test this solution with various reagents for the ions present in it. Testing with various reagents gives characteristic reaction of certain ions, which may be a colour change, a solid formation or any other visible changes.

1.3 Qualitative analysis

Many problems in analytical chemistry begin with the necessity of identifying what is present in a sample. Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are taken from traditional techniques, many of which are still used nowadays. The classical laboratory courses in inorganic and organic qualitative analysis are based on chemical tests to identify inorganic ions (cations and anions) and organic functional groups. The characteristic qualitative analytical reactions are used in the chemical methods of the qualitative analysis. They are characterized by the certain external reactions: precipitates forming with a certain color in solutions, color changes in solution, elimination of evolving gaseous products without odor or with a certain odor, etc. A substance, which is used for carrying out a qualitative analytical reaction, is called a reagent. Each group has a common reagent, which can be used to separate them from others. In these experiments, we will use each ion's unique color, as well as its reactivity to determine the composition of an unknown compound. When a salt, is placed in water, it can dissociate into the ions that comprise it. This experiment will look at some cations such as: Li^+, Na^+, K^+, Ca^{2+}, Sr^{2+}, and Ba^{2+}. The anions Cl^-, Br^-, and I^- will be investigated.

A. Laboratory work presence:

1. Every student must come on time and fill the presence book.
2. Students, who come over than 15 minutes from the laboratory work schedule, may follow the laboratory work if only permitted by the lecturer.
3. All students must participate in every laboratory work activity.
4. The permitted practical tags are twice.

B. Carrying Out of Laboratory Work:

1. Laboratory work of The Practical Textbook of Qualitative and Quantitative Analysis for First Years in Applied Faculties is group laboratory activity. Each group consists of 4 to 5 students. Each group member has individual responsibility to the result of laboratory work (report).
2. Every student has to wear white-laboratory coat, bring a napkin and dropper pipette.

C. Laboratory Work Report:

1. Every student must compose individual report on the student worksheet.
2. Student worksheet must be collected to assistant or lecturer at the same day with laboratory work day.

D. Inside the Laboratory Precautions:

1. Do not eat, drink beverages or chew gum in the laboratory. Do not use laboratory glassware as containers for food or beverages.
2. Wear safety goggles and aprons.
3. Always keep the working area clean and orderly.
4. Know the locations and operating procedures of all safety equipment.
5. Notify the instructor immediately of any unsafe condition you observe.

E. Handling Chemicals:

1. All chemicals in the laboratory are to be considered dangerous. Do not touch, taste or smell any chemical unless specifically instructed to do so.
2. Check the label on chemical bottles twice before removing any of the contents.
3. Never return unused chemicals to their original containers.
4. Acid must be handled with extreme care. Always add acid solely to water.
5. Handle flammable hazardous liquids over a pan to contain spills. Never dispense flammable liquids anywhere near an open flame or source of heat.

F. Handling glassware and Equipment:

1. Always lubricate glassware (tubing, thistle tubes, thermometers, etc.) before attempting to insert it in a stopper.
2. When removing an electrical plug from its socket, grasp the plug, not the electrical cord. Keep your hands dry when working with electricity.
3. Do not immerse hot glassware in cold water, it may shatter.
4. Report damage electrical equipment immediately.

G. Heating Substances:

1. Turn off the gas at gas outlet valve after using.
2. Never leave a lit burner unattended. Never leave anything that is being heated or is visibly reacting unattended.
3. Use tongs or heat-protective gloves when holding or touching heated apparatus.

1.4 Personal and general laboratory safety:

All students must read and understand the information in this document with regard to laboratory safety and emergency procedures prior to the first laboratory session.

1. Never eat, drink, or smoke while working in the laboratory.
2. Read the labels carefully.
3. Do not use any equipment unless you are trained.
4. Wear safety glasses or face shields when working with hazardous materials and/or equipment.
5. Wear gloves when using any hazardous or toxic agent.
6. When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should never be worn in the lab.
7. Keep the work area clear of all materials except those needed for your work.
8. Disposal – Students are responsible for the proper disposal of the material used if any in appropriate containers.
9. If leaving a lab unattended, turn off all ignition sources and lock the doors.
10. Never pipette anything by mouth.
11. Wash hands before leaving the lab and before eating.

1.5 Chemical safety:

1. Treat every chemical as if it were hazardous.
2. Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
3. Never return chemicals to reagent bottles.
4. Use volatile and flammable compounds only in a fume hood.
5. Never allow a solvent to come in contact with your skin. Always use gloves.
6. Never “smell” a solvent!! Read the label on the solvent bottle to identify its contents.

7. Dispose of waste and broken glassware in proper containers.
8. Clean up spills immediately.

1.6 Records of Experiment Laboratory Notebook

All students need to maintain a laboratory notebook. The notebook should be used for the recording of laboratory date and calculations, and critical important for writing your lab reports. The purpose of a laboratory notebook is to allow anyone with some chemical knowledge to understand exactly what you did. You need to record the information in sufficient detail so as to be able to repeat it. And you must be able to understand exactly what your results were. You will need good notes to be able to write your lab reports: in addition, as your understanding of chemistry, improve your notebook may allow you to figures out why some parts of your experiments did not work expected.

1.7 A laboratory notebook should contain many things:

1. A table of contents to aid navigation of notebook.
2. A date on each page.
3. Write information, explanation to yourself of the important of the experiment.
4. Methods notes.
5. Results collected, along with observations.
6. Analysis of the data, tables, graphs and calculations.
7. Brief conclusions.
8. Answer to analysis and comprehension questions for future.

Lab reports are more formal presentation of your results: instead, they focus on clearly. Explaining the significance of the experiment, and clearly worded analysis of the results, leading the reader to conclusion.

Experimental Write-up:

1. Pre- Lab:

1. Introduction
2. Objectives, or purpose
3. Theory

2. Experimental

1. Materials and reagents.
2. List of Equipment's.
3. Flowchart.
4. Record of methods.

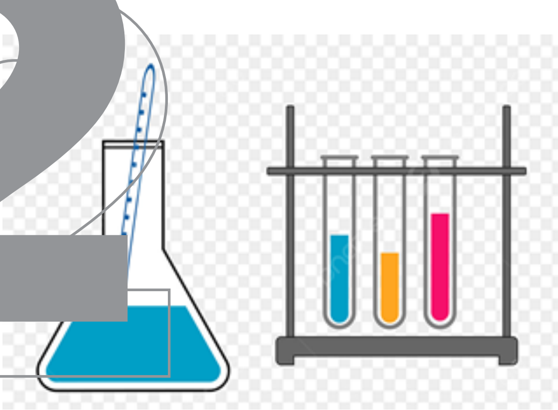
3. Data and calculations.

1. Record of all raw data including printouts.
2. Method of calculation with statistical analysis.
3. Present final data in tables, graphs, or figures when appropriate.

4. Results and Discussion

1. Conclusions.
2. Compare results with known values.
3. Discuss the significance of the data.
4. Was the original objective and Literature references.

Chapter 2



Investigation of Unknown Ions in an Inorganic Salt

Chapter Two

Investigation of Unknown Ions in an Inorganic Salt

2. Systematic Identification of Acidic Radical in inorganic Salts

The qualitative process utilizes reactions characteristic of a given chemical species and interprets the obtained results using a deductive thought process. To facilitate the analysis process, it is often useful to put ions that react in similar ways and follow similar chemical reaction patterns into groups. For instance, some anions such as halides and sulfates are insoluble in lead containing solutions while remaining soluble in iron or copper containing solutions. Therefore, anions can be roughly classified into groups according to their solubility in a particular solution. The reactions used to form groups of anions are referred to in this lab protocol as classification reactions. The individual anions in each group are then separated and identified using different reactions often referred to as confirmatory reactions. The process of determining the identity of an unknown substance, based on a series of chemical tests. Systematic analysis of an inorganic salt involves the following steps:

1. The process of determining the identity of an unknown substance, based on a series of chemical tests.

2. Salts consist of cations (+ve) and anions (-ve).

3. Preliminary examination of solid salt and its solution.
4. Determination of anions by reactions carried out in solution (wet tests) and confirmatory tests.
5. Determination of cations by reactions carried out in solution (wet tests) and confirmatory tests.

In these experiments, the student will learn how to test for inorganic cations and anions, and then identify the contents of unknown salts. Inorganic chemicals have enormous industrial and commercial importance. For example, the single largest chemical produced in the world is sulfuric acid. Therefore, being able to identify and to understand the reactivity of inorganic species is important, and is the topic of this experiment. Naturally, some of these chemical tests are obsolete in terms of identifying unknowns.

Table 2.1: Cation's are positively charged ions:

Monovalent	Divalent	Trivalent
Lithium Li^+ Potassium K^+ Sodium Na^+ Copper(I) Cu^+ Silver Ag^+ Hydrogen H^+	Barium Ba^{2+} Calcium Ca^{2+} Magnesium Mg^{2+} Zinc Zn^{2+} Iron (II) Fe^{2+} Tin (II) Sn^{2+} Lead (II) Pb^{2+} Copper (II) Cu^{2+}	Aluminium Al^{3+} Iron (III) Fe^{3+}

Table 2.2: Solubility and Color of salts

The solubility of a salt can helps us narrow down the possible identity of the salt.

Soluble salts	Insoluble salts
All nitrates, hydrogen carbonates, sodium, potassium and ammonium salts. Most sulphates, chlorides, bromides and iodides. A few carbon-ates and hydroxides.	Most carbonates and hydroxides (except Na^+, K^+ and NH_4^+); $PbSO_4$, $BaSO_4$, $CaSO_4$, AgCl, $PbCl_2$, AgBr, $PbBr_2$, AgI, PbI_2

Table 2.3: the colors of a salt, help the possible identity of the salt.

1. Blue	Cu^{2+} / Co^{2+} salts
2. Green	Cu^{2+} / $Ni2^{+}$ salts
3. Pale Green	Fe^{2+} salts
4. Reddish brown	Fe^{3+} salts / Cu metal
5. Grey	Metals
6. Black	Metal oxides / sulphides i.e., CuO, CuS.

Table 2.4: Known your Laboratory Equipment's

A test tubes: A test tube, also known as a sample tube, is a common piece of laboratory glassware consisting of a finger-like length of glass	
Test tube brush: Test tube brush or spout brush is a brush used for cleaning test tubes and narrow mouth laboratory glassware, such as graduated cylinders, burettes, and Erlenmeyer flasks.	
Test tube holder: A test tube holder is used to hold test tubes. It is used for holding a test tube in place when the tube is hot or should not be touched.	
Test tube stand: Test tube racks are laboratory equipment used to hold upright multiple test tubes at the same time.	
Tongs: Tongs are a type of tool used to grip and lift objects instead of holding them directly with hands.	

Wash Bottle: A wash bottle is a squeeze bottle with a nozzle, used to rinse various pieces of laboratory glassware, such as test tubes and round bottom flasks. Wash bottles are sealed with a screw-top lid.	
Fusion tube: Fusion tube is laboratory tube used much in the same way as boiling tubes expect not being as large and thick walled. A fusion tube made of thinner glass because it is intended to be broken into a container of water at the end of the fusion tube.	
Graduated cylinder: Graduated cylinders are used to transfer liquids with a moderate degree of accuracy.	

2.1. Experiment No. 1: Investigation of unknown anions in inorganic Salts Group I:

2.1.1 Preliminary Test with Dilute HCl Acid Reagent

Theory: any group of unknown elements has specific reagent. In this test the action of dilute Hydrochloric acid on the salt is noted at room temperature and on warming. The following tests for the following anions are fully described including explanations, methods, observations and equations, so described are how to test for the following anions – negative ions: carbonate ion CO_3^{2-}, hydrogen carbonate ion HCO_3^-,sulphite (IV)/sulfite(IV) ion SO_3^{2-}, sulphide /sulfide ion S^{2-}, Theory: any group of unknown elements has specific reagent. In this test the action of dilute Hydrochloric acid on the solid salt is noted at room temperature and on warming. CO_3^{-2}, HCO_3^-, SO_3^{-2}, $S_2O_3^-$ and NO_2^-react with dilute hydrochloric acid to evolve different gases. Study of the characteristics of the gases evolved gives information about the anions.

Objective:

1. Identify the presence of each of the following anions: SO_3^{2-}, $S_2O_3^{2}$, S_2^{-} CO_3^{2}- HCO_3^{-}, NO_2^{-}, and using qualitative analysis.
2. Identify the anions in an unknown sample of ionic salts utilizing the information gathered in Part I. And to answer the following questions.

Apparatus:

Test tubes, Test tubes holder, glass rod, beakers, sample bottles, Bensn burner.

Physical properties of salt.

1. Exp. No
2. Date
3. Title
4. Color
5. Shape
6. Solubility:

Methods

Take 0.1 g of salt in a test tube and 3-4 drops of dilute acid added to a portion of the unknown solid and look for effervescence. Bubble the gas formed. Add the reagent of group to the solid salt into test tube.

Table 2.4: Steps of investigation of unknown anions in inorganic Salts of group (I) Dilute HCl Acid Reagent

Group Reagent	Observation	Results
Preliminary tests		
Salt solution +HCl	Colorless, colorless gas with brisk effervescence which turn lime water milky.	Anions
Dilute HCl	a) Colorless, odorless gas with brisk effervescence (CO_2) which turn lime water milky. b) Colorless gas with rotten egg like smell (H_2S) which turns lead acetate paper black. c) Colorless gas with smell of burning sulphur (SO_2) which turns acidified dichromate paper green. d) Brown colored gas (NO_2) which turns ferrous sulphate solution black or brown. e) Colorless gas with vinegar like smell.	CO_3^{2-} S^{2-} SO_3^{2-} NO_2^{-}
Confirmative tests		
Salt+ $BaCl_2$ Solution Salt+ $MgSO_4$ solution	White ppt of $BaCO_3$ White ppt of $MgCO_3$	CO_3^{2-} CO_3^{2-}
	a) Colorless pungent smelling gas (HCl) which gives white dense fumes with glass rod dipped in NH_4OH. b) Violet colored vapours (I_2) which turns starch paper blue. c) Reddish brown gas (NO_2) having pungent smell (On adding copper turning, fumes become intense) d) Brown color gas with pungent smell (Br_2) which turns starch paper yellow.	Cl- I- NO_3- Br-
$BaCl_2$	White ppt. of $BaSO_4$ is formed	SO_4^{2-}, PO_4^{3-}

Equations:

1. $Na_2CO_3 + 2HCl \rightarrow 2NaCl + CO_2 + H_2O$
2. $CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O$
3. $NaHCO_3 + HCl \rightarrow NaCl + CO_2 + H_2O$
4. $CO_3^{2-}{}_{(s)} + 2H^+_{(aq)} \rightarrow H_2O_{(l)} + CO_{2(g)}$
5. $HCO_3^-{}_{(s)} + H^+_{(aq)} \rightarrow H_2O_{(l)} + CO_{2(g)}$

If CO_2 gas is passed in excess through lime water, the milkiness produced disappears due to the formation of calcium hydrogen carbonate which is soluble in water.

1. $CaCO_3 + CO_2 + H_2O \rightarrow Ca(HCO_3)_2$
2. $2NaHCO_3 + MgSO_4 \rightarrow Mg(NaHCO_3)_2 + Na_2SO_4$

Gas liberated:

1. CO_2 Carbonate/Combustion of fuel or
2. H_2 Metal - Acid Reaction
3. O_2 Nitrates/Oxides of less reactive metals/Hydrogen peroxide
4. NH_3 Ammonium salts
5. Cl_2 Chlorides / Chlorates
6. SO_2 Sulphites / Sulphates

Tests for Gases

1. **Carbon dioxide**: Bubble the gas into a tube of aqueous calcium hydroxide (limewater). If the gas forms a white precipitate with limewater, the gas is carbon dioxide gas.
2. **Sulfur dioxide:** Dip a piece of filter paper into aqueous acidified potassium manganite (VII) and insert the paper into a tube of the gas. If the gas turns purple acidified potassium manganate (VII) colorless (if the gas decolorizes purple acidified potassium manganite (VII), the gas is sulfur dioxide gas.

Table 2.5: Preliminary examinations with concentrated HCl acid

Observations	Gas Evolved	Possible Anion
A colorless, odorless gas is evolved with brisk effervescence, which turns lime water .milky	CO_2	CO_3^{2-}

Colorless gas with the smell of rotten eggs is evolved which turns lead acetate paper .black	H_2S	S^{2-}
Colorless gas with a pungent smell, like burning sulphur which turns acidified potas-.sium dichromate solution green	SO_2	SO_4^{2-}
Brown fumes which turn acidified potassium iodide solution containing starch solution .blue	NO_2	NO_3^-

Equations:

1. $Na_2S + HCl \rightarrow NaCl + H_2S$
2. $(CH_3COO)_2Pb + H_2S \rightarrow PbS + CH_3COOH$
3. $Na_2SO_3 + 2HCl \rightarrow 2NaCl + H_2O + SO_2$
4. $Na_2S_2O_3 + 2HCl \rightarrow 2NaCl + H_2O + S + SO_2$
5. $NaNO_2 + HCl \rightarrow NaCl + HNO_2$
6. $2HNO_2 \rightarrow H_2O + NO + NO_2$

2.1.2 Test for Carbonate ions (CO_3^{2-}, HCO_3^-)

All carbonates react with dilute acids (HCl) to form salt, water and CO_2 gas. Add dilute acid to a portion of the unknown solid and look for effervescence. Bubble the gas formed into limewater. If the gas is CO_2, a white ppt is formed with limewater (aq calcium hydroxide), this indicates the presence of carbonate ion. The gas turns lime water milky due to the formation of $CaCO_3$.

Confirmatory tests: If CO_2 gas is passed in excess through lime water, the milkiness produced disappears due to the formation of calcium hydrogen carbonate which is soluble in water.

1. $CaCO_3 + CO_2 + H_2O \rightarrow Ca\ (HCO_3)_2$
2. $2NaHCO_3 + MgSO_4 \rightarrow Mg\ (NaHCO_3)_2 + Na_2SO_4$

HCO_3^{-2} Bicarbonate: Carbonate solution gives Wight precipitate with magnesium sulphate directly and after heating with bicarbonate.

1. $2NaHCO_3 + MgSO_4 \rightarrow Mg(NaHCO_3)_2 + Na_2SO_4$
2. $2Mg(HCO_3)_2 \rightarrow MgCO_3 + H_2O + CO_2$
3. $MCO_3 + MgSO_4 \rightarrow M_2SO_4 + MgCO_3$
4. $Na_2CO_3 + MgSO_4 \rightarrow Na_2SO_4 + MgCO_3$
5. $4Na_2CO_3 + 4HgCl_2 \rightarrow Hg_4O_3CO_3 + 8NaCl + 3CO_2$

2.1.3 Test for Sulphide ion (S^{2-})

With warm dilute HCl gives hydrogen sulphide gas which smells like rotten eggs. A piece of filter paper dipped in lead acetate solution turns black on exposure to the gas due to the formation of lead sulphide which is black in color.

1. $Na_2S + 2HCl \rightarrow NaCl + H_2S$
2. $(CH_3COO)_2Pb + H_2S \rightarrow PbS + CH_3COOH$

Confirmatory tests:

1. $Na_2S + AgNO_3 \rightarrow Ag_2S + NaNO_3$
2. $Na_2S + Pb(CH3CO_2)_2 \rightarrow PbS + 2CH_3COOH$

2.1.4 Test for Sulphite ion ($SO_3^{\ 2-}$)

In treating sulphite with warm dil. H_2SO_4, SO_2 gas is evolved which is suffocating with the smell of burning sulphur.

1. $Na_2SO_3 + 2HC2 \rightarrow NaCl + H_2O + SO_2$
2. $Na_2S_2O_3 + 2HCl2 \rightarrow NaCl + H_2O + S + SO_2$

The gas turns potassium dichromate paper acidified with dil. H_2SO_4, green.

$$K_2Cr_2O_7 + H_2SO_4 + 3SO_2 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + H_2O$$

Chromium sulphate (green)

Confirmatory tests: With silver nitrate solution.

1. $Na_2SO_3 + AgNO_3 \rightarrow 2NaNO_3 + Ag_2SO_3$
2. $Ag_2SO_3 + Na_2SO_3 \rightarrow 2Ag[AgSO_3]$
3. $Na_2SO_3 + Pb(CH_3CO_2)_3 \rightarrow 2NaCH_3CO_2 + PbO$

2.1.5 Test for Nitrite ion (NO_2^-)

On treating a solid nitrite solid with dil. HCl and warming, reddish brown fumes of NO_2 gas are evolved. Addition of potassium iodide solution to the salt solution followed by freshly prepared starch solution and acidification with acetic acid produces blue color.

1. $NaNO_2 + HCl \rightarrow NaCl + HNO_2$
2. $2HNO_2 \rightarrow H_2O + NO + NO_2$

Confirmatory tests: With solution of KI:

1. $2KNO_2 + 2KI + 2H_2SO_4 \rightarrow K_2SO_4 + 2NO_2 + 2H_2O + I_2$
2. $5NaNO_2 + 2KI + 3H_2SO_4 + KMnO_4 \rightarrow 2NaNO_3 + K_2SO_4 + 2MnSO_4 + 2H_2O$

2.1.6 Brown Ring Nitrate test

Method: Perform the brown ring test for nitrates on a sample of aqueous $NaNO_3$. Be careful! Put 20 drops of the solution to be tested into a test tube, and then, carefully and slowly, add 20 drops of concentrated H_2SO_4. If necessary, cool this mixture by allowing cold tap water to run over the outside of the test tube. In a second test tube, dissolve about 0.1 g $FeSO_4.7H_2O$ in 1 mL of water. Now, hold the test tube containing the H_2SO_4 solution at a 45^o angle while you allow 5 drops of the $FeSO_4$ solution to run slowly down the inside of the tube. The aqueous $FeSO_4$ should form a layer above the acid. If nitrate ion is present, a smoky brown ring will form at the solution interface. This ring may take several minutes to form, and will disappear eventually.

1. $2NaNO_2 + H_2SO_4 \rightarrow 2HNO_2 + Na_2SO_4$
2. $3HNO_2 \rightarrow 2NO + HNO_3 + H_2O$
3. $2FeSO_4 + NO \rightarrow Fe(NO)SO_4$

2.1.7 Test for Thiosulphate $S_2O_3^{-2}$

1. $Na_2S_2O_3 + 2HCl + 3H_2SO_4 \rightarrow 2NaCl + H_2O + SO_2 + S$
2. $SO_2 + K_2Cr_2O_7 + 3H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 3H_2O$

Confirmatory tests:

1. with silver nitrate solution:

1. $Na_2S_2O_3 + AgNO_3 \rightarrow 2NaNO_3 + Ag_2S_2O_3$
2. $Ag_2S_2O_3 + H_2O \rightarrow Ag_2S + H_2SO_4$

2. with barium chloride solution:

$Na_2S_2O_3 + BaCl_2 \rightarrow BaS_2O_3 + 2NaCl$

$Na_2SO_3 + BaCl_2 \rightarrow BaSO_3 + 2NaCl$

$BaSO_3 + HCl \rightarrow BaCl_2 + H_2O + SO_4^{-2}$

2.2 Experiment No. 2: Investigation of Conc H_2SO_4 Group Anions

2.2.1 Preliminary Test with Concentrated H_2SO_4 Reagent

If no positive result is obtained from using dil. HCl test, take 0.1 g of solid salt in a dry clean test tube and 3-4 drops of conc. H_2SO_4. Observe the change in the reaction mixture in cold and then warm it. Identify the gas evolved on heating. Identify the presence of each of the following anions: Cl^-, Br^-, I^- , and NO_3^-, using qualitative analysis.

Apparatus: Test tubes glass rod, beakers, sample bottles.

Physical properties of salt.

1. Exp. No
2. Date
3. Title
4. Color
5. Solubility

Methods:

Table 2.6: Preliminary examinations with concentrated H_2SO_4

Observations	Gas	Results
A colorless gas with pungent smell, which gives dense white fumes when a rod dipped in ammonium hydroxide, .is brought near the mouth of the test tube	HCl	Choloride
Reddish brown gas with a pungent odor is evolved. Intensity of reddish gas increases on heating the reaction mixture after addition of solid MnO_2 to the reaction mixture. .Solution also acquires red color	Br_2 vapors	Bromide
Violet vapors, which turn starch paper blue and a layer of violet sublimate is formed on the sides of the tube. Fumes .become dense on adding MnO_2 to the reaction mixture	I_2 vapors	Iodide
Brown fumes evolve which become dense upon heating the reaction mixture after addition of copper turnings and .the solution acquires blue color	NO_2	Nitrate

Equations:

1. $2NaCl + H_2SO_4 \rightarrow Na_2SO_4 + 2HCl$
2. $2HCl + NH_3 \rightarrow NH_4Cl$
3. $2KBr + H_2SO_4 \rightarrow K_2SO_4 + 2HBr$
4. $2HBr + H_2SO_4 \rightarrow SO_2 + 2H_2O + Br_2$
5. $KI + H_2SO_4 \rightarrow K_2SO_4 + 2HI$
6. $2HI + H_2SO_4 \rightarrow SO_2 + 2H_2O + I_2$
7. $2KNO_3 + H_2SO_4 \rightarrow K_2SO_4 + 2HNO_3$
8. $Cu + 4HNO_3 \rightarrow Cu(NO_3)_2 + 2NO_2 + 2H_2O$

2.2.2 Confirmatory tests of Chlorine and Iodine

Chlorides:

1. Chloride ions react with aqueous silver nitrate to form a white ppt which is insoluble in dilute nitric acid, but soluble in aqueous ammonia.

2. To a portion of the solution of the unknown, add dilute nitric acid followed by aqueous silver nitrate. Formation of a white ppt indicates the presence of chloride ions.

1. $Ag^+(aq) + Cl^-(aq) \rightarrow AgCl(s)$

If on treatment with warm conc. H_2SO_4 the salt gives a colorless gas with pungent smell or and if the gas which gives dense white fumes with ammonia solution, then the salt may contain Cl^- ions and the following reaction occurs.

1. $NaCl + H_2SO_4 \rightarrow NaHSO_4 + HCl$
2. $HCl + NH_3 \rightarrow NH_4Cl$ Ammonium chloride White fumes
3. $2NaCl + Pb(CH_3CO_2)_2 \rightarrow PbCl_2 + 2CH_3COON$

Iodides:

1. Iodide ions react with aqueous silver nitrate / aqueous lead (II) nitrate to form a yellow ppt of silver iodide or lead (II) iodide respectively. The ppt is insoluble in both dilute nitric acid and aqueous ammonia.
2. To a portion of the solution of the unknown, add dilute nitric acid followed by aqueous silver nitrate / aqueous lead (II) nitrate. Formation of a yellow ppt indicates the presence of iodide ions.

1. $Ag^+(aq) + I^-(aq) \rightarrow$ 1. $AgI(s)$
2. 2. $PbI_2(s) \rightarrow Pb^{2+}(aq) + 2I^-(aq)$
3. 3. $2NaI + Pb(CH_3CO_2)_2 \rightarrow PbI_2 + 2CH_3COONa$
4. 4. $NaI + AgNO_3 \rightarrow AgI + NaNO_3$

2.2.3 Steps of investigation of SO_4^{2-} and PO_4^{3-}

After the students have studied the experiment, they should be able to: Investigation of Anions: Identify the presence of each of the following anions: SO_4^{2-} PO_4^{3-}, using qualitative analysis.

Chemicals:

1. $BaCl_2$
2. $(CH_3COO)_2Pb$
3. $NaNO_2$
4. $Ca(OH)_2$
5. $FeSO_4$

Apparatus: Test tubes glass rod, beakers, sample bottles.

2.2.4 Test for Sulphate and Phosphate ions

If no positive test is obtained in Steps-I and II, then tests for the presence of sulphate and phosphate ions are performed.

Table 2.7: Confirmatory examinations

Ion	Confirmatory test
SO_4^{2-}	a) Take 1 mL water extract of the salt in water or sodium carbon-) ate and after acidifying with dilute hydrochloric acid add $BaCl_2$ solution. White precipitate insoluble in conc. HCl or conc. HNO_3 .is obtained b) Acidify the aqueous solution or sodium carbonate extract with) acetic acid and add lead acetate solution. Appearance of white .precipitate confirms the presence of SO_4^{2-} ion
PO_4^{3-}	Acidify sodium carbonate extract or the solution of the salt in water with conc. HNO_3 and add ammonium molybdate solution .and heat to boiling. A canary yellow precipitate is formed

2.2.5 Confirmatory Test of Sulphate ions (SO_4^{2-})

(a) Aqueous solution addition of barium chloride gives a white precipitate of barium sulphate insoluble in conc. HCl or conc. HNO_3.

1. $Na_2SO_4 + BaCl_2 \rightarrow BaSO_4$ (white ppt) + 2NaCl

(b) Sulphate ions give white precipitate of lead sulphate when aqueous solution or sodium carbonate extract neutralized with acetic acid is treated with lead acetate solution.

2. Na_2SO_4 + $(CH_3COOO)_2Pb \rightarrow PbSO_4$ (white ppt) + $2CH_3COONa$

2.2.6 Test for Phosphate ion (PO_4^{3-})

(a) Add conc. HNO_3 and ammonium molybdate solution to the test solution containing phosphate ions and boil. A yellow coloration in solution or a canary yellow precipitate of ammonium-phosphomolybdate, $(NH_4)_3[P(Mo_3O_{10})_4]$ is formed. Each oxygen of phosphate has been replaced by Mo_3O_{10} group.

$$Na_2HPO_4 + 12(NH_3)MoO_4 + 23HNO_3 \rightarrow (NH_4)_3[P(Mo_3O_{10})_4] + 2NaNO_2 + 21NH_4NO_3 + 12H_2O$$

2.3 Exp. No. 3 Investigation of Qualitative Analysis of Cations

Goal: In this experiment you will explore characteristic reactions of a number of cations. The reactions you observe will be used to identify unknown salts. We will use this study to develop logical schemes for analyzing unknowns, and also to learn and review some descriptive chemistry of the elements. Qualitative analysis involves the identification of what is in an unknown. It is extremely useful to know how to detect the presence of specific ions. The basis for a qualitative analysis is the fact that ions will undergo specific chemical reactions with certain reagents to yield observable products. Careful notebook records should be kept of all tests on knowns and unknowns. Results in qualitative analysis are easily confused, and it is therefore very important that you be diligent about labeling your various test tubes, and that you record your observations immediately.

Physical Properties: Some preliminary tests need to be done before doing the analysis of cations. Look carefully at the sample. Colors and consistency can tell you something about the compound. The physical examination of the unknown salt involves the study of colour, smell and density. The test is not much reliable, but certainly helpful in identifying some colour cations. Characteristic smell helps to identify some ions like ammonium ion. The following cations will often have the following colors. Alkali and alkaline metal salts are usually colorless

1. Cr^{+3} deep green; (Cr^{+6}) bright orange/yellow

2. Mn^{2+} very faint blush pink
3. Fe (+2) pastel green; (+3) yellow to brown
4. Co^{2+} purple or brown
5. Ni^{2+} pale green
6. Cu^{2+} blue if ligated (e.g., with water), white otherwise

Material Required:

1. Boiling tube
2. Test tubes
3. Measuring cylinder
4. Test tube stand
5. Test tube holder
6. Delivery tube
7. Corks
8. Filter paper

Table 2.8: Identification of Cations (Basic Radicals)

G. No.	Reagent	PPt	References
1	Dilute HCl	chlorides	Ag^+,Hg_2^+, Pb^{2+}
2	H_2S gas in presence of dil. HCl	Sulfides	a) Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+} b) Sb^{3+}, Sb^{5+}, Sn^{2+}, Sn^{4+}
3	NH_4OH in presence of NH4Cl	Hydroxides	Al^{3+}, Fe^{2+}/Fe^{3+}, Cr^{3+}
4	H_2S in presence of NH4OH	Sulfides	Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}
5	$(NH_4)_2CO_3$ in presence of NH_4OH	Carbonates	Ca^{2+}, Sr^{2+}, Ba^+
6			NH_4^+, Na^+, K^+, Mg^{2+}

2.3.1 Wet Tests for Identification of Cations

The cations indicated by the preliminary tests given above are confirmed by systematic analysis given below. The first essential step is to prepare a clear and transparent solution of the salt. This is called **original solution**. It is prepared as follows: **Preparation of Original Solution:** to prepare the original solution, following steps are followed one after the other in a systematic order. In case the salt does not dissolve in a particular solvent even on heating, try the next solvent.

The following solvents are tried:

1. Take a little amount of the salt in a clean boiling tube and add a few mL of distilled water and shake it. If the salt does not dissolve, heat the content of the boiling tube till the salt completely dissolves.

2. If the salt is insoluble in water as detailed above, take fresh salt in a clean boiling tube and add a few ml of dil. HCl to it. If the salt is insoluble in cold, heat the boiling tube till the salt is completely dissolved.

3. If the salt does not dissolve either in water or in dilute HCl even on heating, try to dissolve it in a few ml of conc. HCl by heating.

4. If salt does not dissolve in conc. HCl, then dissolve it in dilute nitric acid.

5. If salt does not dissolve even in nitric acid, then a mixture of conc. HCl and conc. HNO_3 in the ratio 3:1 is tried. This mixture is called aqua regia. A salt not soluble in aqua regia is considered to be an insoluble salt.

2.4 Experiment No. 4 Analysis of Group I: Pb^{2+}, Hg_2^{2+}, Ag^+

Many medicines, which contain compounds of silver, lead and mercury cations, are used in medical practice because they possess antiseptic properties. Silver (I) nitrate aqueous solutions (w=1-2%) are applied as antiseptic agents for treating eye and skin diseases. Lead oxide PbO is a basic active substance of leaden plaster; it is used for skin festering and inflammatory diseases. Mercury (I) chloride (calomel) is used as a diuretic and purgative. It should be remembered that mercury and its salts are ***very toxic***!

2.4.1 Preliminary Tests Group I Cations

This group includes Pb^{2+}, Ag^+, Hg_2^{2+}. Here we shall study only Pb^{2+}. Group reagent for this group is dil. HCl. The addition of HCl to the solution will precipitate Pb^{2+} as lead chloride which is soluble in hot water. On cooling, the precipitate settles down as $PbCl_2$ which is less soluble in cold water. The precipitating reagent of Group I is a dilute solution of hydrochloric acid, and the ions precipitated are those of silver, Ag^+, mercury (I), Hg_2^{2+}, and lead (II), Pb^{2+}. The net ionic equations are:

1. $Ag^+ + Cl- \rightarrow AgCl_{(s)}$ (AgCl is white, turning purple in light)
2. $Hg_2^{2+} + 2Cl^- \rightarrow Hg_2Cl_2(s)$ (Hg_2Cl_2 is white)
3. $Pb^{2+} + 2Cl^- \rightarrow PbCl_{2(s)}$ ($PbCl_2$ is white)

Table 2.9 Confirmatory and Confirmative tests of group I

Experiment	Preliminary Test		
	^+Ag	Pb^{2+}	Hg_2^{2+}
Salt solution + HCl	White ppt of AgCl Purple insoluble in acids but soluble in HCl	white ppt of $PbCl_2$ soluble in hot water	White ppt of $Hg_2Cl_{2,}$ not insoluble in diluted acids and converted to black in presence of NH_4OH
Confirmative Tests			
Salt solution + KI	Yellow ppt of AgI insoluble in acids but soluble in NH_4OH	Yellow ppt of PbI_2 soluble in addition reagent	Yellow greenish ppt of Hg_2I_2 soluble in addition reagent
Salt solution + K_2CrO_4	Brick reddish ppt of Ag_2CrO_4 insoluble in diluted HNO_3 of NH_4OH	yellow ppt of $PbCrO_4$ soluble in diluted HNO_3 but insoluble NH_4OH	Brown ppt of Hg_2CrO_4 when heated converted ot crystal red

$PbCl_2 + H_2O \rightarrow Pb^2{}_{+(aq)} + 2Cl^-$

$Pb^{2+} + CrO_4^{2-} \rightarrow PbCrO_4$

Take a small amount of original solution (if prepared in hot conc. HCl) in a test tube and add cold water to it and cool the test tube under tap water. If a white precipitate appears, this indicates the presence of Pb^{2+} ions in group –I. On the other hand, if the original solution is prepared in water and on addition of dil. HCl, a white precipitate appears, this may also be Pb^{2+}.

2.4.2 Confirmatory tests of Lead (II) ion (Pb^{2+})

1. Potassium iodide test

Lead chloride (formed by the reaction of lead salts with dil. HCl) solution in hot water reacts with potassium iodide solution to form yellow precipitate of lead iodide, obtained which confirms the presence of Pb^{2+} ions.

$PbCl_2 + 2KI \rightarrow PbI_2 + 2KCl$

(b) Potassium chromate test

Lead chloride (formed by the reaction of lead salts with dil. HCl) solution in hot water reacts with solution of potassium chromate (K_2CrO_4) solution to form yellow precipitate of lead chromate. This confirms the presence of Pb^{2+} ions.

$PbCl_2 + K_2CrO_4 \rightarrow PbCrO_4 + 2KCl$

The yellow precipitate ($PbCrO_4$) is soluble in hot NaOH solution.

$PbCrO4 + 4NaOH \rightarrow Na_2[Pb(OH)_4] + Na_2CrO_4$

Sodium tetra hydroxoplumbate (II)

2.5 Experiment No. 5 Analysis of Group II Cations

If group-I is absent, add excess of water to the same test tube. Warm the solution and pass H_2S gas for 1-2 minutes. Shake the test tube. If a precipitate appears, this indicates the presence of group-II cations. Passing of H_2S gas through the acidified original solution will precipitate the radicals as their sulphides. Pass more H_2S gas through the solution to ensure complete precipitation and separate the precipitate. If the color of the precipitate is black, it indicates the presence of Cu^{2+} ions. If it is yellow in color, then presence of As^{3+} ions is indicated. Take the precipitate of group-II in a test tube and add excess of yellow ammonium sulphide solution to it. Shake the test tube. If the precipitate is insoluble, **group II-A (**Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}**)** is present. If the precipitate is soluble, this indicates the presence of **group-II B (**Sb^{3+}, Sb^{5+}, Sn^{2+}, Sn^{4+}**)**. Medicines, which contain compounds of copper (II), mercury (II) cations, are used in medical practice. Copper (II) sulphate is used as an antiseptic, astringent or cauterizing agent as solutions (w=0.25

%) in eye practice and urology. Mercury (II) oxide is used in ophthalmology and dermatology as oinments. It should be remembered that mercury and its salts are very toxic! But in the large doses the compounds of all these metals are very poisonous. This group includes.

A. Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}

B. Sb^{3+}, Sb^{5+}, Sn^{2+}, Sn^{4+}

Table 2.10: Confirmatory tests for Group-II A and II B cations

Experiment	Preliminary Test	
	Observations	Interference
Salt solution + dilute HCl	Black ppt of Group II A ions (Bi^{3+}, Cu^{2+}, Cd^{2+}) insoluble in yellow $(NH_4)_2S$ is formed	Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}
	If a black ppt soluble in yellow $(NH_4)_2S$ is formed then As^{3+} ion is present.	Bi^{3+}, Cu^{2+}, Cd^{2+}
Confirmative Tests		
Experiments	Observations	Results
Salt solution + dilute HNO_3	ppt insoluble in HNO_3 acid but soluble in NH_4OH	Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}
Salt solution + NaOH	Pale greenish ppt insoluble in addition excess reagent	Cu^{2+}
Salt solution + NaOH	White ppt insoluble in addition excess reagent	Cd^{2+}
Salt solution + NaOH	Brown ppt convert to yellow for forming HgO	Hg^{2+}
Salt solution + NH_4OH	White ppt insoluble in additional reagent	Hg^{2+}
Salt solution + NH_4OH	Blue greenish ppt soluble in NH_4OH	Cu^{2+}
Salt solution + KI	No ppt formed	Cd^{2+}
Salt solution + KI	White ppt with brown color	Cu^{2+}

Salt solution + KI	Red ppt insoluble addition excess KI	Hg^{2+}
Salt solution + KI	Black ppt soluble in addition excess KI to form yellow orange	Bi^{3+}

2.5.1 Confirmatory tests of Group-II A cations

Copper (II) ion (Cu^{2+})

1. $CuSO_4 + Na_2S \rightarrow CuS + Na_2SO_4$
2. $3CuS + 8HNO_3 \rightarrow 3Cu(NO_3)_2 + 2NO + 3S + 4H_2O$
3. $S + 2HNO_3 \rightarrow H_2SO_4 + 2NO$
4. $2Cu^{2+} + SO_4^{2-} + 2NH_3 + 2H_2O \rightarrow Cu(OH)_2.CuSO_4 + 4\ 2NH_4^+$
5. $Cu(OH)_2.CuSO_4 + 8NH_3 \rightarrow 2[Cu(NH_3)_4]SO_4 + 2OH^- + SO_4^{2-}$
6. $2Cu\ SO_4 + 2KI \rightarrow CuI_2 + K_2SO_4$
7. $CuI_2 \rightarrow Cu_2I_2 + I_2$

Bismuth:

1. $Bi(NO_3)_2 + Na_2S \rightarrow Bi_2S_3 + 2NaNO_3$
2. $Bi(NO_3)_2 + 3NaOH \rightarrow Bi(OH)_3 + 3NaNO_3$
3. $Bi(NO_3)_2 + 3NH_4OH \rightarrow Bi(OH)_3 + 3NH_4NO_3$
4. $Bi(NO_3)_2 + 3KI \rightarrow BiI_3 + 3KNO_3$

Mercury:

1. $HgCl_2 + Na_2S \rightarrow HgS + 2NaCl$
2. $HgCl_2 + 2NaOH \rightarrow Hg(OH)_2 + 2NaCl$
3. $Hg(OH)_2 + heat \rightarrow HgO + H_2O$
4. $HgCl_2 + 2NH_4OH \rightarrow Hg(NH_2) + NH_4Cl + H_2O$

Lead

1. $Pb(NO_3)_2 + Na_2S \rightarrow PbS + 2NaNO_3$

2. $Pb(NO_3)_2 + 2NaOH \rightarrow Pb(OH)_2 + 2NaNO_3$

3. $Pb(NO_3)_2 + 2NH_4OH \rightarrow Pb(OH)_2 + 2NH_4NO_3$

4. $Pb(NO_3)_2 + 2KI \rightarrow PbI_2 + 2KNO_3$

Confirmatory tests of Arsenic (III) ion (As^{3+}): The yellow residue of As_2S_3 formed in the group analysis is dissolved in conc. HNO_3 forming arsenic acid.

2.6 Experiment No. 6 Analysis of Group – III Cations

Many medicines, which contain compounds of aluminum, lead and mercury cations, are used in medical practice because they possess antiseptic properties. Aluminium hydroxide in the form of suspensions in the mixture with magnesium hydroxide (almagel) is used for treating gastritis and gastric ulcer as an anti-acidic, absorbent and covering agent. Alums $KAl(SO_4)_2 \times 12H_2O$ are applied as a hemostatic agent. Chromium, arsenic, tin as microelements play a very important biological part in the human's organism. But in the high doses metals and their compounds are very poisonous. The cations present in this group are Fe^{2+}, Fe^{3+}, Cr^{3+} and Al^{3+}. We will look at only Fe^{2+}/ Fe^{3+} and Al^{3+}. The cations in this group are precipitated as ***hydroxides*** by adding ammonium hydroxide in presence of ammonium chloride. Thus, group reagent for this group is NH_4OH in the presence of NH_4Cl.

Table 2.11: Confirmatory and Preliminary tests for Group III

Experiment	Preliminary Test	
	Observations	Interference
Salt solution + NaOH + NH_4OH+ access NH_4Cl	The reddish brown ppt	Fe^{2+}/Fe^{3+}, $Fe^{3+} + 3NaOH \rightarrow 2Fe(OH)_3 + 3Na^+$
Salt solution + NaOH + NH_4OH+ access NH_4Cl	White gelatinic ppt soluble in mineral acids and NaOH	Al^{3+} $Al^{3+} + 6NaOH \rightarrow 2Al(OH)_3 + 6Na^+$ $2Al(OH)_3 + NaOH \rightarrow 2Al(OH)_3 + H_2O$

Salt solution + NaOH + NH_4OH+ access NH_4Cl	Green greyish ppt soluble in mineral acids and NaOH	Cr^{3+} $Cr^{3+} + nNH_4OH \rightarrow nCr(OH)_3 + nNH_4^+$
Confirmative Tests		
Methods	Observations	Results
Add Salt solution + Na_2HPO_4	The White yellowish ppt insoluble in acetic acid, but soluble in mineral acids. Fe^{3+} +3NaOH $2Fe(OH)_3$ + $3Na^+$	Fe^{3+}, $Fe^{3+} + 2Na_2HPO_4 \rightarrow FePO_4 + 3Na^+ + Na_2HPO_4$
Salt solution + Na_2HPO_4	White gelatin ppt soluble in mineral acids, NaOH insoluble in acetic acid,	$Cr^{3+} + 2Na_2HPO_4 \rightarrow CrPO_4 + 3Na^+ + NaH_2PO_4$
Salt solution + Na_2HPO_4	Gerry greenish ppt soluble in mineral acids,	$Al^{3+} + 2Na_2HPO_4 \rightarrow AlPO_4 + 3Na^+ + NaH_2PO_4$
Salt solution + SCN	No ppt formed	Fe^{3+}, Cr^{3+}, Al^{3+}
Salt solution + SCN	Solution color converted from red blood.	Fe^{3+}, $Fe^{3+} + 6SCN \rightarrow Fe[SCN]_6]^{3-}$
Salt solution + Sodium acetate CH_3COONa	White ppt after heating	Al^{3+} $Al^{3+} + CH_3COONa \rightarrow Al(OH)_2(C_2H_3CO_2)$
Salt solution + Sodium acetate CH_3COONa	White ppt after heating	Fe^{3+} $Fe^{3+} + CH_3COONa \rightarrow Fe(OH)_2(C_2H_3CO_2)$
Salt solution + Sodium acetate CH_3COONa	No change happens	Fe^{3+} or Cr^{3+}

Equations of cations o Group III:

1. $FeCl_3 + 3NH_4OH \rightarrow Fe(OH)_3 + 3NH_4Cl$
2. $FeCl_3 + 3NaOH \rightarrow Fe(OH)_3 + 3NaCl$
3. $Fe^+ + 2Na_2HPO_4 \rightarrow FePO_4 + 3Na^+ + NaH_2PO_4$
4. $FeCl_3 + 3CH_3CO_2Na \rightarrow (CH_3CO_2)_3Fe + 3NaCl$
5. $(CH_3CO_2)_3Fe + H_2O \rightarrow Fe(OH)_2\ CH_3COO + 2CH_3COOH$

Aluminium Salts

1. $AlCl_3 + 3NH_4OH \rightarrow 2Al(OH)_3 + 3(NH_4)_2SO_4$
2. $Al_2(SO_4)_3 + 6NaOH \rightarrow 2Al(OH)_3 + 3Na_2SO_4$
3. $2Al(OH)_3 + NaOH \rightarrow NaAlO_2 + H_2O$

Chromium Salts

1. $CrCl_3 + 3NH_4OH \rightarrow Cr(OH)_3 + 3NH_4Cl$
2. $Cr^{+3} + 3OH$- $Cr(OH)_3\downarrow$ grey-green
3. $CrCl_3 + 3NaOH \rightarrow Cr(OH)_3 + 3NaCl$
4. $Cr(OH)_3 + NaOH \rightarrow NaCrO_3 + H_2O$
5. $CrCl_3 + 2Na_2HPO_4 \rightarrow CrPO_4 + 3NaCl + NaH_2PO_4$

2.7 Experiment No. 7 Analysis of Group – IV Cations

The Goal

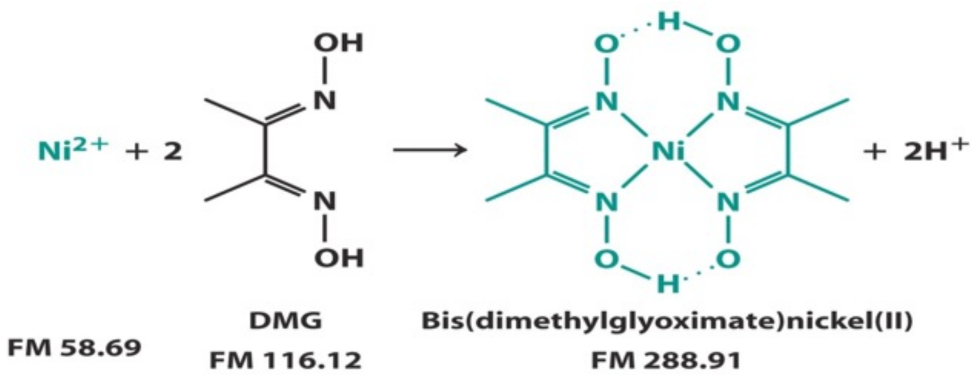
Zinc sulphate suppresses reproduction of micro-organisms. Therefore, zinc sulphate solutions (w=0.25 % and w=0.3 %) are applied in medical practice as eye drops. Zinc oxide ZnO is used in dermatology as ointments and powders as an astringent and antimicrobial agent. The ratio-active cobalt of ^{60}Co is applied in medicine for treating malignant tumors. Cobalt as vitamin B_{12} takes part in the synthesis of hemoglobin. Nickel as a microelement also takes part in very important biological processes in the human organism.

The radicals present in this group are Co^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+}. These are precipitated as sulphide by passing H_2S gas through the ammonical solution of the salt. The group reagent for this group is **H_2S** gas in the presence of NH_4Cl and NH_4OH. Passing of H_2S gas through the group III solution will precipitate the radicals Co^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} as their sulphides. Formation of black ppt. (CoS or NiS) indicates cobalt or nickel. Formation of buff-coloured ppt. (MnS) indicates manganese and dirty white ppt. (ZnS) indicates zinc.

Table 2.12: Preliminary and Confirmative Tests for Group IV

Methods	Preliminary Test	
	Observations	Results
Add Salt solution + H_2S + NH_4OH+ NH_4Cl	black ppt. (CoS or NiS)	Ni^{2+} Co^{2+} $Ni^{2+} + Na_2S \rightarrow NiS + 2Na^+$ $Co^{2+} + Na_2S \rightarrow CoS + 2Na^+$
Salt solution + H_2S + NH_4OH+ NH_4Cl	Buff-coloured ppt. (MnS) indicates manganese	Mn^{3+} $Mn^{2+} + Na_2S \rightarrow MnS + 2Na^+$
Salt solution + H_2S + NH_4OH+ NH_4Cl	Dirty white ppt. (ZnS) indicates zinc.	Zn^{2+} $Zn^{2+} + Na_2S \rightarrow ZnS + 2Na^+$
Confirmative Tests		
Methods	Observations	Results
Salt solution + NH_4OH	The White gelatine ppt insoluble in access reagent	Zn^{2+} $Zn^{2+} + 2NH_4OH \rightarrow Zn(OH)_2 + 2NH_4^+$
Salt solution + NH_4OH	The White ppt converted to brown insoluble in access reagent	Mn^{2+} $Mn^{2+} + 2NH_4OH \rightarrow Mn(OH)_2 + 2NH_4^+$
Salt solution + NH_4OH	Green ppt insoluble in access reagent	Ni^{2+} $Ni^{2+} + 2NH_4OH \rightarrow Ni(OH)_2 + 2NH_4^+$
Salt solution + NH_4OH	Blue ppt soluble in access reagent	Co^{2+} $Co^{2+} + 2NH_4OH \rightarrow Co(OH)_2 + 2NH_4^+$

Salt solution + NaOH	White ppt convert to soluble in access reagent	Zn^{2+} $Zn^{2+} + 2NaOH \rightarrow Zn(OH)_2 + 2Na^+$
Salt solution + NaOH	White ppt convert to brown insoluble in access reagent	Mn^{2+} $Mn^{2+} + 2NaOH \rightarrow Mn(OH)_2 + 2Na^+$
Salt solution + NaOH	Green ppt soluble in access reagent	Ni^{2+} $Ni^{2+} + 2NaOH \rightarrow Ni(OH)_2 + 2Na^+$
Salt solution + NaOH	Blue ppt convert to pink by addition access reagent	Co^{2+} $Co^{2+} + 2NaOH \rightarrow Co(OH)_2 + 2Na^+$
Salt solution + NaCN, SCN	No ppt	Ni^{2+}, Mn^{2+}, n^{2+} $Co^{2+} + 2NaOH \rightarrow Co(OH)_2 + 2Na^+$
Salt solution + NaCN, SCN	Blue color soluble in emyly alcohol	Co^{2+}, $Co(cn)_2 + 4SCN \rightarrow [Co(SCN)_4]^{2+}\ \ 2Na^+$
Salt solution + Dimethylglyoxime	No ppt	Co^{2+}, Mn^{2+}, Zn^{2+}
Salt solution + Dimethylglyoxime	Red ppt	Ni^{2+},



2.8 Experiment No. 8 Analysis of Group V Cations

Group V consist of three radicals: Ba^{2+}, Sr^{2+} and Ca^{2+}. These cations are precipitated as their carbonates. Group reagent for this group is $(NH_4)_2CO_3$ in the presence of NH_4Cl and NH_4OH. When $(NH_4)_2CO_3$ is added to salt solution containing NH_4Cl and NH_4OH, the carbonates of Ba^{2+}, Sr^{2+} and Ca^{2+} are precipitated.

Table 2.13: Preliminary and Confirmative Tests for Group V

Experiment	Preliminary Test	
	Observations	Interference
Salt solution + NH_4Cl + drops NH_4OH+$(NH_4)_2CO_3$	White ppt. soluble in mineral and acetic acids	Ca^{2+} $Ca^{2+} + (NH_4)_2CO_3 \rightarrow CaCO_3 + 2NH_4^+$
Salt solution + NH_4Cl + drops NH_4OH+$(NH_4)_2CO_3$	White ppt. soluble in HCl	Sr^{2+} $Sr^{2+} + (NH_4)_2CO_3 \rightarrow SrCO_3 + 2NH_4^+$
Salt solution + NH_4Cl + drops NH_4OH+$(NH_4)_2CO_3$	White ppt. soluble in mineral and acetic acids	Ba^{2+} $Ba^{2+} + (NH_4)_2CO_3 \rightarrow BaCO_3 + 2NH_4^+$
Confirmative Tests		
Experiments	Observations	Interference
Salt solution + $CaSO_4$	No ppt formed	Ca^{2+}
Salt solution + $CaSO_4$	White ppt formed slowly increase by heat	Sr^{2+} $Sr^{2+} + CaSO_4 \rightarrow SrSO_4 + Ca^{2+}$
Salt solution + $CaSO_4$	White ppt immediately soluble in minerals acid and insoluble in acetic acid	Ba^{2+} $Ba^{2+} + CaSO_4 \rightarrow BaSO_4 + Ca^{2+}$

Salt solution + $(NH_4)_2C_2O_4$	White ppt immediately soluble in minerals acid and insoluble in acetic acid	Ca^{2+} $Ca^{2+} + (NH_4)_2C_2O_4 \rightarrow CaC_2O_4 + 2NH_4^+$
Salt solution + $(NH_4)_2C_2O_4$	White ppt after heat soluble in minerals acid	Sr^{2+} $Sr^{2+} + (NH_4)_2C_2O_4 \rightarrow SrC_2O_4 + 2NH_4^+$
Salt solution + H_2SO_4 conc	White ppt soluble in $(NH_4)_2SO_4$ conc	Ca^{2+} $Ca^{2+} + H_2SO_4 \rightarrow CaSO_4 + 2H^+$
Salt solution + H_2SO_4 conc	White ppt soluble condense insoluble in mineral acids and $(NH_4)_2SO_4$ conc	Sr^{2+} $Sr^{2+} + H_2SO_4 \rightarrow SrSO_4 + 2H^+$
Salt solution + H_2SO_4 conc	White ppt soluble condense immediately insoluble in mineral acids $(NH_4)_2SO_4$ concentrated.	Ba^{2+} $Ba^{2+} + H_2SO_4 \rightarrow BaSO_4 + 2H^+$
Salt solution + CrO_4^{2+}	yellow ppt insoluble in acetic acid	Ba^{2+} $Ba^{2+} + CrO_4^{2+} \rightarrow BaCrO_4 + 2H^+$
Salt solution + CrO_4^{2+}	No ppt formed	Ca^{2+}
Salt solution + CrO_4^{2+}	yellow ppt soluble in acetic acid	Sr^{2+} $Sr^{2+} + CrO_4^{2+} \rightarrow SrCrO_4 + 2H^+$

2.9 Experiment No. 9 Analysis of Group VI Cations

Chemical compounds of the **6st** analytical group cations are widely used in medical practice. For example, 0.9% aqueous solution of NaCl is used to maintain the blood plasma volume. Sodium bicarbonate is used to maintain the acid-base balance. KCl is a drug that is used to treat arrhythmias. KI is a source of iodine when there is the lack of it in the body. 10% Ammonia solution is used for breathing stimulation. Ammonia chloride is used as a diuretic for treating cardiac hypostases. K+, Na+, NH4⁺ form compounds with the ionic type of the chemical bond and most of them are readily soluble in water.

2.9.1 General characteristic of cations K^+, Na^+, NH4⁺

K^+, Na^+ and NH_4^+ hydrolyzed ions are colorless. Salts of these ions have color if there are painted anions in their composition. For example, Na_2CrO_4 is yellow, $KMnO_4$ is violet. K^+ and Na^+ compounds easily form oversaturated solutions. That is why for acceleration of their precipitates crystallization it is necessary to rub the inner wall of a test tube where the reaction proceeds with a glass stick.

Method:

Pour 3-5 drops of the aqueous solution of potassium salt into a small test tube; add 3 drops of tartaric acid and sodium acetate and rub the inner wall side of test tube by a glass stick. Soon a white crystalline precipitate will be formed. Divide the precipitate into two test tubes and dissolve the first part of the precipitate in an acid and the second part in a base.

1. Analysis of (NH_4^+ ion)

(a) Take 0.1 g of salt in a test tube and add 1-2 mL of NaOH solution to it and heat. If there is a smell of ammonia, this indicates the presence of ammonium ions. Bring a glass rod dipped in hydrochloric acid near the mouth of the test tube. White fumes are observed.

(b) Pass the gas through **Nessler's** reagent. Brown precipitate is obtained. The ammonium ion, NH_4^+ is the conjugated acid of the basic ammonia, NH_3. The test for NH_4^+ takes advantage of the following equilibrium.

$NH_4^+ + 2OH^- \rightarrow NH_3 + H_2O$

Thus, when this strong base is added to the solution of ammonium salt and this solution is heated, NH_3 gas is evolved. Gaseous ammonia can be determined: by odor; and by coloring of a humid red litmus paper in a dark blue color. Litmus paper should not touch test tube walls to prevent hit of alkali on the paper.

2. Confirmatory Tests for NH^+_4 ion

(a) Ammonia gas evolved by the action of sodium hydroxide on ammonium salts reacts with hydrochloric acid to give ammonium chloride, which is visible as dense white fume.

$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_3 + 2H_2O$

$NH_3 + HCl \rightarrow NH_4Cl$

On passing the gas through Kessler's reagent, a brown coloration or a precipitate of basic mercury (II) amido-iodine is formed.

$2K_2HgI_4 + NH_3 + 3KOH \rightarrow HgO.Hg(NH_2)I + 7KI + 2H_2O$

Basic mercury (II) amido-iodine (Brown precipitate)

The ammonium ion, NH_4^+ is the conjugated acid of the basic ammonia, NH_3. The test for NH_4^+ takes advantage of the following equilibrium:

$NH_4^+ + 2OH^- \rightarrow NH_3 + H_2O$

2.9.2 Flame Test

The flame test is a method used in chemistry to detect the presence of certain metal ions, based on each element's characteristic emission spectrum. The color of flames in general also depends on temperature. The flame test is fast and easy to perform, and does not require any equipment not usually found in a chemistry laboratory. However, the range of detected elements is small, and the test relies on the subjective experience of the experimenter rather than any objective measurements. The flame test is fast and easy to perform, and does not require any equipment not usually found in a chemistry laboratory. However, the range of detected elements is small, and the test relies on the subjective experience of the experimenter rather than any objective measurements. To perform the flame test, take a piece of a platinum or nichrome wire sealed in a piece of glass tubing. The chlorides of several metals impart characteristic color to the flame because they are volatile in non-luminous flame.

Table 2.14: shows some colors of cations flame test

Color of the flame observed	Color of the flame observed	Results
Green flame with blue	Same color	Cu^{2+}
crimson	purple	Sr^{2+}
Apple green	Blush green	Ba^{2+}
Brick red	green	Ca^{2+}

This test is performed with the help of a platinum wire as follows:

Method:

1. Make a tiny loop at one end of a platinum wire.
2. To clean the loop dip, it into concentrated hydrochloric acid and hold it in a non-luminous flame.
3. Repeat step (ii) until the wire imparts no color to the flame.
4. Put 2-3 drops of concentrated hydrochloric acid on a clean watch glass and make a paste of a small quantity of the salt in it.
5. Dip the clean loop of the platinum wire in this paste and introduce the loop in the non-luminous (oxidizing) flame.
6. Observe the color of the flame first with the naked eye and then through a blue glass and identify the metal ion with the help of Table below. Inference from the flame test:

For proceeding to this test see the following figure 2.2., the paste of the mixture with conc. HCl is introduced into the flame using a platinum wire.


Barium - Green


Barium - Green


Barium - Green


Barium - Green


Barium - Green


Barium - Green

Figure 2.2: Described who the flame test Method

Chapter

3

Volumetric Analysis

Chapter Three

Volumetric Analysis

3. Introduction of volumetric analysis

Titrimetric analysis involves determination of the volume of a solution of accurately known concentration, which is required to react quantitatively with the measured volume of the solution of a substance, concentration of which is to be determined. The solution of accurately known concentration is called **standard solution**. The mass of the substance dissolved in the solution of unknown concentration is calculated from the volume of the standard solution used, the chemical equation and the relative molecular masses of the reacting compounds. The reagent of known concentration is called **titrant** and the substance being titrated is termed as **titrant**.

To carry out titrimetric analysis, standard solution is usually added from the long-graduated tube called burette. The process of adding the standard solution to the solution of unknown concentration until the reaction is just complete is called **titration**. The point at which reaction is completed is called **equivalence point** or the **theoretical or stoichiometric end point**. Titration is the process of determining how much of a material is present in a known volume of a substance. Titration may be defined as the process of changing the color of a substance that has been dissolved in a liquid and then determining the concentration of the substance based on the color change.

Types of titrations:

1. acid-base reaction
2. Oxidation Reduction reaction
3. Precipitation
4. Complex formation (EDTA) ethylenediaminetetraacetic acid.

1. **$HCl + NaOH \rightarrow H_2O + NaCl$**
2. **$5NaC_2O_4 + 2KMnO_4 + 6H^+ \rightarrow 10CO_2 + 2Mn^{2+} + 8H_2O$**
3. **$Ag^+ + X^- \rightarrow AgX(s)$**
4. Complex formation (EDTA) ethylenediaminetetraacetic acid.

3.1 Standard Solution

A solution of exactly known concentration is called standard solution. Any substances, which are stable at room temperature and do not, react with solvent, in which it is dissolved, can be directly weighed to prepare its standard solution. Description and preparation of these solutions is given below:

$$\textbf{N of HCl Concentration} = \frac{(\text{Density} \times \text{perentage} \times 1000)}{(\text{Equ.Wt})}$$

$$12\text{N of HCl} = \frac{1.18 \times 0.37 \times 1000}{36.5}$$

VN = VN

How do you prepare 250mL of HCl =0.1N?

$$\textbf{Wt. in gm} = \frac{\text{M x M.wt x V}}{1000}$$

3.2 Primary and secondary standards

A **primary standard** is a compound of sufficient purity in which total amount of impurities does not exceed 0.01-0.02%. The standard solution can be prepared by direct weighing of a sample of primary standard followed by its dissolution in water (or solvent) to obtain a definite volume of solution. The substance to be used as a primary standard should also satisfy the following requirements:

1. It must be easily available in pure and dry form.
2. It should not undergo change in air i.e., it should not be hygroscopic, oxidized by air or affected by gases such as carbon dioxide present in the atmosphere or lose water of crystallization, so that it can be stored safely.
3. It should be easy to detect the impurities present in it.
4. It should have high relative molecular mass.
5. Its reaction with another substance should be stoichiometric.
6. The substance should be readily soluble in water.

Notes: Primary standards salts such as" (potassium hydrogen phthalate (M.wt 204.22, potassium hydrogen iodate, 389.91, sodium carbonate: 106 and Borax: 381.37)

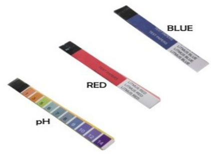
Percent Purity Calculations: Weigh accurately a portion of the impure sample and dissolve it in an arbitrary amount of solvent.

$$\text{Purity \%} = \frac{M \times M.wt \times V}{1000} \times 100$$

Figure 3.1 Glass and Apparatus of different size and Known your Laboratory Equipment's

Beakers: Beakers are used as containers. They are available in a variety of sizes. Although they often possess volume markings, these are only rough estimates of the liquid volume. The markings are not necessarily accurate. Like beakers, laboratory flasks can be made of glass or plastics.	
Graduate cylinders: It is a primary measuring tool for the volume of a liquid. There are several markings up and down the length of the container with specific .increments	

Graduated cylinders: Are used to measure the volume of liquids precisely, ranging from a few milliliters to several liters. These laboratory vessels are essential for mixing or dispensing different liquids and for the preparation of solutions with pre-defined concentrations .and volume	
Digital Balance: The digital mass balances in the General Chemistry labs are very sensitive instruments used for weighing substances to the milligram (0.0001 g) level. Use containers when weighing chemicals and .always weigh objects at room temperature	
Volumetric flask: Volumetric flasks are used to measure and store solutions with a high degree of accuracy. These flasks generally possess a marking near the top that indicates the level at which the volume of the liquid .is equal to the volume written on the outside of the flask	
Pipette: Pipettes are used for transferring liquids with a fixed volume and quantity of liquid must be known to a .high degree of accuracy	
Burette clamp: Burette clamp is scientific equipment which used specifically to hold and secure a burette on a stand, so that a burette is fixed and more convenient for the experiment. Burette clamp can be made by many .materials such as plastic and cast iron	
Funnel: A tube or pipe that is wide at the top and narrow at the bottom, used for guiding liquid or powder into a .small opening	
Round-bottom flasks: Round-bottom flasks (also called round-bottomed flasks) are types of flasks having spherical bottoms used as laboratory glassware, mostly .for chemical or biochemical work	

Mortar and pestle: Mortar and pestle are implements used since ancient times to prepare ingredients or substances by crushing and grinding them into a fine .paste or powder in the laboratory	
Litmus and pH paper: Litmus or pH paper contains a chemical that changes color as it makes contact with an acid or base. The paper will turn red in acids and blue in .bases	
Crucible: A crucible is a ceramic or metal container in which metals or other substances may be melted or .subjected to very high temperatures	
Water bath: A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature .over a long period of time	
Digital pH meter: A pH meter is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as .pH	
Digital Conductometer: A conductivity meter measures the electrical conductivity in a solution. It has multiple .applications in research and engineering	
Burette: an instrument used to measure volume; a graduated glass tube about 40 cm long with a stopcock on one end. The volume measurement is made by reading the fluid level in the burette before and after the **titrant**, the fluid in the burette is dispensed through the .stopcock	

3.3 Titration elements:

1. ***Titration*** is an analytical method used to determine the exact amount of a substance by reacting that substance with a known amount of another substance. The completed reaction of a titration is usually indicated by a color change or an electrical measurement.
2. ***Standard solution**:* a solution of known concentration.
3. ***Unknown**:* a substance or a mixture about which something is not known.
4. ***Indicators:*** Indicators are chosen, such that they change colors at the range of the pH of interest. The solution itself at the end-point may be: Basic, if the reaction involves a strong base and a weak acid. Neutral, if the reaction involves a strong acid and a strong base.
5. ***Equivalence point:*** The point at which there are stoichiometrically equivalent amounts of acid and base. $[H^+] = [OH^-]$
6. ***End point**:* the stage in the titration at which the indicator color change is observed, indicating that the reaction is complete. The following are titration and acid-base neutralization.

3. 4 Experiment No. 1 Determination of the NaOH Using Standard Solution of HCl (0.1M)

An acid/base neutralization reaction will yield salt and water. In an acid-base titration, the neutralization reaction between the acid and base can be measured with either a color indicator or a pH meter.

Objective: Determination of the concentration (strength) of a given sodium hydroxide solution by titrating it against a standard solution of hydrochloric acid.

Theory

In the titration of a strong acid with a strong base, the amount of acid and base becomes chemically equivalent at the end point and the chemical reaction is called neutralization reaction. Near the end point there is a sudden change in the pH of the solution. If after end point even a small amount of base/acid is added the solution would become slightly alkaline or acidic respectively. In this experiment, a phenolphthalein color indicator will be used. Phenolphthalein (ph.ph) is colorless in acidic solutions and pink in basic solutions. Phenolphthalein is also used in forensic crime scene analysis to detect the presence of blood, Kastle-Meyer test. How do you found the concentration of NaOH solution by using standard HCl solution? Find the concentration of NaOH: mol/liter and in g/liter?

Equation: Acid Base Neutralization Reaction

1. Acid + Base Water + Salt
2. Ex: HCl + NaOH H_2O + NaCl

Chemicals: NaOH solution unknown concentration, HCl (0.1M) standard solution and Ph.ph indicator.

Apparatus: Burette, Pipette, Conical flask, funnel, stand, beakers.

Method:

A laboratory method for determining the concentration of an unknown acid or base using a neutralization reaction. A *standard solution*, (a solution of known concentration), is used. Acidic, if the reaction involves a strong acid and a weak base. a). Using stoichiometry b) using the titration formula $aM_aV_a=bM_bV_b$

1. Fill the 50ml capacity of pipette with the HCl acid solution above the calibration mark. To obtain an accurate reading, you should have the calibration mark at eye level; i.e., your line of sight should be parallel with the mark.
2. Transfer the 10.00 ml of the NaOH solution into the clean 250 ml Erlenmeyer flask. Add 2-3 drops of phenolphthalein indicator to Erlenmeyer flask.
3. Close the stopcock and wait a few seconds for drainage to be complete, then note the reading on the burette to two decimal places ($V_{initial}$).

4. Titrate to the end point against standard solution from the burette to two decimal places (V_{final}). Repeat the above process (three times).

Results

Table 3.2 Determination of the of NaOH solution using standard HCl (0.1M)

.No	($V_{initial}$ (ml	(V_{final} (ml	(V_{used} (ml	End-point

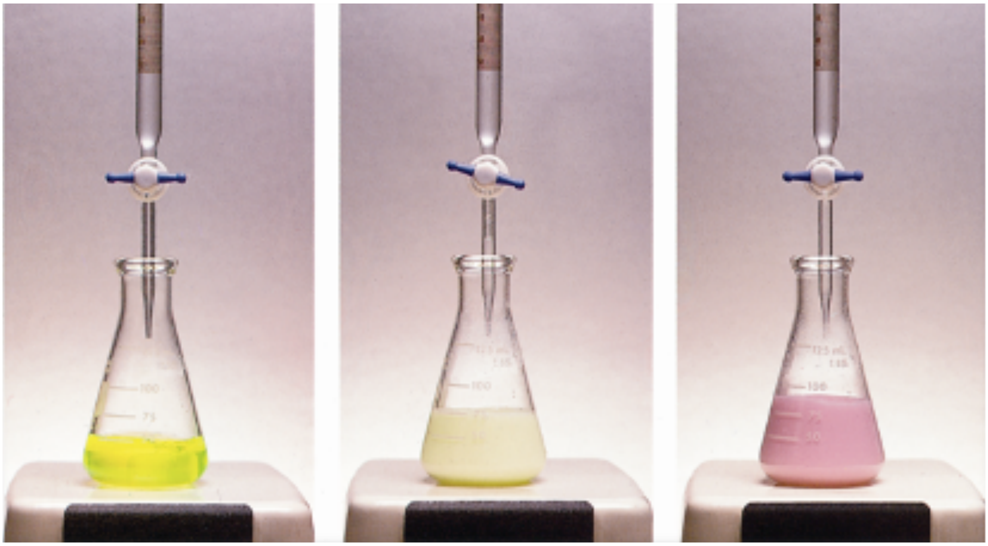


Fig. 3.1 shows the gradually of coloration of neutralization

3.5 Experiment No. 2 Determination Concentration of Na_2CO_3 Using Standard HCl

Goal: Determination of the strength of a given solution of dilute hydrochloric acid by titrating it against a standard solution of sodium carbonate.

Theory

Preparation of 0.1 M standard solution of sodium carbonate. Sodium carbonate has characteristics nearer to the primary standards therefore its standard solution can be made by direct weighing. To prepare 0.1 M Na_2CO_3 solution, 10.6000g of sodium carbonate should be dissolved per liter of the solution (Molar mass of sodium carbonate is 106 g mol^{-1}). Therefore, to prepare 100 ml of 0.1M Na_2CO_3 solution 1.0600 g of sodium carbonate is dissolved in minimum quantity of water and the solution is diluted to exactly 100 ml by adding water to it. The strength of HCl is determined by titrating it against a standard solution of sodium carbonate. In this titration, methyl orange, a weak base (yellow in the unionized form) is used as an indicator. In this experiment also, the titration follows the usual course, i.e., the proton furnished by the addition of the acid first neutralizes sodium carbonate solution. When the entire sodium carbonate solution is neutralized, the last drop of the acid added from the burette produces the pinkish red color change, which is the end point. The concentration (strength) of the unknown solution is calculated in g/L. It is calculated from the molarity of the solution.

Here, the molarity equation is written as Base Acid

$$a1M_1V_1 = a2M2V_2$$

where, a1 and a2 are the acidity and basicity of the alkali and the acid respectively. *M*1 and *M*2 are the molarities, *V*1 and *V*2 are the volumes of the base and acid respectively used to neutralise each other.

Objectives: Find the concentration of Na_2CO_3. In mol/L and g/L.

1. $Na_2CO_3 + HCl \rightarrow NaCl + \cancel{HNaCO_3}$
2. $HNaCO_3 + HCl \rightarrow NaCl + H_2O + CO_2$

---

3. $\cancel{Na_2CO_3} + 2HCl \rightarrow 2NaCl + H_2O + CO_2$

Apparatus:

1. Burette (50 mL)
2. Pipette (10 mL)

3. Conical flask (100 mL)
4. Burette stand
5. Funnel
6. Glazed tile (white)
7. Measuring flask (100 mL)

Chemicals: Na_2CO_3 solution unknown concentration, HCl (0.1M) standard solution and Methyl orange indicator.

Method:

1. Fill the 50mL pipette with HCl (0.1M) acid solution above the calibration mark.
2. Transfer the 10.00 ml of the Na_2CO_3 solution into the clean 250 mL Erlenmeyer flask. Add 2-3 drops of phenolphthalein indicator to Erlenmeyer flask.
3. Turn and rotate the burette so all inside surfaces have come into contact with the base solution.
4. Close the stopcock and wait a few seconds for drainage to be complete, and then note the reading on the burette to two decimal places ($V_{initial}$).
5. Place the Erlenmeyer flask under the tip of burette and put the white background under the Erlenmeyer flask. Titrate to the end point against standard solution and take the volume of burette to two decimal places (V_{final}). As shown in Figure 3.5.
6. Repeat the above process (three times) to take average.

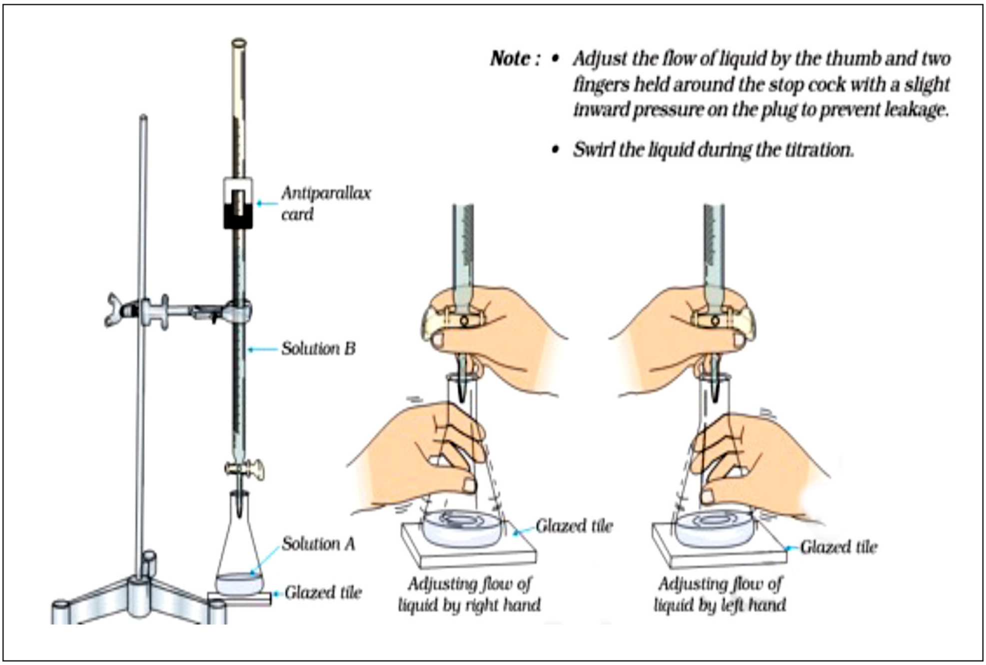


Figure 3.2 how can use the titration equipment's

3.6 Experiment No. 3 Determination of concentration NaOH using standa rd solution of oxalic acid

Goal and theory: In the titration between oxalic acid (weak acid) and sodium hydroxide (strong base), following reaction takes place:

$H_2C_2O_4 + 2NaOH \rightarrow NaC_2O_4 \ +H_2O$

In this titration phenolphthalein is used as an indicator. The concentration of unknown solution is calculated in g/L. Molarity of the solution can be calculated by using the formula

$a1\ M_1\ V_1 = a2\ M_2\ V_2$

where a1, M_1, V_1 are respectively basicity, molarity and volume of acid used and a2, M_2 and V_2 are acidity, molarity and volume respectively of base used in the titration.

Material: Oxalic acid, Sodium hydroxide solution and Phenolphthalein indicator

Method:

(A) Preparation of 0.1M Standard Solution of Oxalic Acid

(B) Titration of Sodium Hydroxide and Oxalic Acid Solution

1. Clean the burette thoroughly, wash it with distilled water and finally rinse it with sodium hydroxide solution. Clamp the burette vertically in a burette stand.
2. Fill sodium hydroxide solution into the burette through a funnel above the zero mark.
3. Remove the air gap, if any, from the nozzle of the burette by running the solution forcefully from the burette nozzle.
4. Remove the funnel before noting initial reading of the burette.
5. Note the initial reading by keeping the eye exactly at the same level as the meniscus of the solution.
6. Pipette out 10 ml of oxalic acid solution in a washed and dried conical flask. Always wash the pipette with water and rinse with the liquid to be measured before pipetting out the liquid.
7. Add 1-2 drops of ph.ph indicator to the conical flask.
8. Titrate the acid with sodium hydroxide solution till a very faint permanent pink color is obtained. Add sodium hydroxide solution in small amounts initially and then drop wise.
9. Read the lower meniscus of the solution in the burette again and record it as final reading.
10. Repeat the procedure until three concordant readings are obtained. Record your readings.

Table 2.2: Titration of sodium hydroxide vs oxalic acid solution

No	V of oxalic taken in conical flask each time V 1mL	Burette readings		Concordant reading in mL
1		Initial reading (x)	Final reading (y)	
2				
3				

Molarity of NaOH solution can be calculated by using the equation: Oxalic acid Sodium hydroxide a1 M1V1 = a2 M2V2 where, M1 and V1 are the molarity and volume of the oxalic acid solution. M2 and V2 are the molarity and volume of the sodium hydroxide solution. a1 and a2 are respectively the basicity of oxalic acid and acidity of sodium hydroxide. In this case a1= 2 and a2 = 1. Also, Molar mass of oxalic acid, $(COOH)_2.2H_2O$ = 126 g mol–1 and Molar mass of sodium hydroxide (NaOH) = 40 g mol–1

Calculations: Calculate the concentration of sodium hydroxide solution in g/L by using the equation given below.

Concentration (strength) in g/L = Molarity × Molar mass.

Result Concentration of NaOH solution is ———————— g/L.

3.7 Experiment No. 4 Analysis of Strong and Weak Bases Mixture by using Strong acid

Find the molar concentration for each sodium hydroxide and sodium carbonate solution mixtures using standard solution of hydrochloric acid (0.1M).

Method:

1. Clean the burette using distilled water and fill with hydrochloric acid (0.0M).
2. Pipette 10ml of mixture of sodium hydroxide and carbonate solution in conical flask.

3. Add 2 -3 drops of ph.ph indicator. The volume of hydrochloric acid consumed reacts with all hydroxide and half of carbonate solution mixture.
4. Titrate against standard solution of hydrochloric acid (0.0M) till the pink color appears.
5. Repeat the above steps using Methyl orange indicator. The volume of hydrochloric acid consumed reacts with all hydroxide and all carbonate solution mixture.
6. Calculation the concentration in molar and g/L of mixtures contents?

Table 2.3: Titration of hydrochloric acid (0.1M) NaOH and Na_2CO_3 mixture

No	using ph.ph	Burette readings		V1 of HCl ml
1		Initial reading (x)	Final reading (y)	
2				
3				
	using Methyl Orange	Initial reading (x)	Final reading (y)	V2 of HCl ml
1				
2				
3				

3.8 Experiment No.5 Determination of Concentration of Sulphuric Acid using NaOH and Standard HCl Solutions (0.1M)

Objective:

Determination of the concentration (strength) of a given sluphuric acid by titration using sodium hydroxide solution which titrated with against a standard solution of hydrochloric acid (0.1M). For determination of end point by reaction of standard hydrochloric acid with sodium solution using Ph.ph indicator according to the following equation.

Theory

1. $HCl + NaOH \ \ H_2O + NaCl$

For determination of end point of H2SO4 reaction with sodium hydroxide solution using methyl orange indicator according to following equation.

2. $H_2SO_4 + NaOH \ \ \ Na_2SO_4 + H_2O$

Chemicals:

1. NaOH solution unknown concentration.
2. HCl (0.1M) standard solution.
3. Sulphuric acid unknown concentration.
4. Methyl orange indicator.
5. Ph.ph indicator.

Apparatus: Burette, Pipette, Conical flask, funnel, stand, beakers.

Method

A laboratory method for determining the concentration of an unknown acid or base using a neutralization reaction. A *standard solution*, (a solution of known concentration), is used. Acidic, if the reaction involves a strong acid and a weak base.

I. Using stoichiometry
II. using the titration formula $aM_aV_a \ \ bM_bV_b$

Part I: Using Ph.ph indicator

1. Fill the 50ml capacity of pipette with the HCl acid solution above the calibration mark. To obtain an accurate reading, you should have the calibration mark at eye level; i.e., your line of sight should be parallel with the mark.

2. Transfer the 10ml of the NaOH solution into the clean 250 ml Erlenmeyer flask. Add 2-3 drops of phenolphthalein indicator to flask content.
3. Close the stopcock and wait a few seconds for drainage to be complete, then note the reading on the burette to two decimal places ($V_{initial}$).
4. Titrate to the end point against standard solution from the burette to two decimal places (V_{final}). Repeat the above process (three times).

Results

Table 3.4 Determination of the of NaOH solution using standard HCl (0.1M)

No.	$V_{initial}$ (ml)	V_{final} (ml)	V_{used} (ml)	End-point

Part II: Using methyl orange indicator

1. Fill the 50ml capacity of pipette with the H_2SO_4 acid solution above the calibration mark. To obtain an accurate reading, you should have the calibration mark at eye level; i.e., your line of sight should be parallel with the mark.
2. Transfer the 10ml of the NaOH solution into the clean 250 ml Erlenmeyer flask. Add 2-3 drops of methyl orange indicator to flask content.
3. Close the stopcock and wait a few seconds for drainage to be complete, note the reading on the burette to two decimal places ($V_{initial}$).
4. Titrate to the end point against standard solution H_2SO_4 acid from the burette to two decimal places (V_{final}).
5. Repeat the above process (three times).

Table 3.5 Determination of the of H_2SO_4 acid solution using standard NaOH

No.	$V_{initial}$ (ml)	V_{final} (ml)	V_{used} (ml)	End-point

Conclusion:

I. The concentration of H_2SO_4 acid?

II. Strength of H_2SO_4 acid in g/L?

3.9 Experiment No. 6 Analysis of Borax solution by using Standard Hydrochloric Acid

Find the molar concentration and the strength of borax solution using standard solution of hydrochloric acid (0.05M).

Theory: $Na_2B_4O_7$+ 2HCl + .5H_2O à 2NaCl + 3H_3PO_3

Chemicals:

1. Borax solution unknown concentration.
2. HCl (0.1M) standard solution.
3. NaOH solution unknown concentration.
4. Methyl orange indicator.
5. Ph.ph indicator.
6. This experiment is designed to enhance your skill in the precise use of volumetric analysis.

Theory: Borax $Na_2B_4O_7 10H_2O$ can be used as primary standard solution, its readily obtained in very pure form 99.99% purity. It reacts with a known stoichiometry and can be weighed and used directly in water the salt dissociates:

$Na_2B_4O_7 10H_2O$ 2Na+ + $B_4O_7^{-2}$ + 10H_2O

And anion is hydrolyzed as:

$B_4O_7^{-2} + 7H_2O \ 4H_3BO_3 + 2OH^-$

The liberated of OH^- can be titrated with strong HCl and neutralized the solution contain H_3BO_3 acid which is dissociate as:

$H_3BO_3 + H_2O \ H_2BO_3^- + H_3O^+$

Method

Weigh accurately 0.15g of borax into 150mL conical flask. Dissolve the sample in 25mL of water, warm gently if necessary and titrate with HCl (0.05M) from the burrate using M.O and ph,ph indicators. Calculate the concentration of borax solution in g/L.

3.10 Oxidation Reduction Experiments

Objectives

- ✓ Learn and reinforce the concepts of titration as an analytical method
- ✓ Develop skills with substances that may be hazardous
- ✓ Gain experience with stoichiometry in balanced reactions and observe a reduction oxidation process

Oxidation–reduction reactions are widely used in qualitative analysis for detection of ions. In order to identify drugs different group of redox reactions, which have a characteristic color or other analytical features, are used. In the quantitative analysis oxidation-reduction reactions are the basis of redox processes. Oxidation-reduction reactions are of great importance in biological systems. Photosynthesis, breathing, digestion — all these are chains of oxidation–reduction reactions. In engineering, the significance of redox reactions is also very great. For example, the entire metallurgical industry is based on oxidation–reduction processes during which metals are recovered from natural compounds.

3.10.1 Redox Titration

1. Redox titrations are based on a reduction-oxidation reaction between an oxidizing agent and a reducing agent. A potentiometer or a redox indicator is usually used to determine the endpoint of the titration, as when one of

the constituents is the oxidizing agent potassium dichromate. The color change of the solution from orange to green is not definite; therefore, an indicator such as sodium diphenylamine is used.

2. Some redox titrations do not require an indicator, due to the intense color of the constituents. For instance, in ***permanganometry*** a slight faint persisting pink color signals the endpoint of the titration because of the color of the excess oxidizing agent potassium permanganate.

3.6.2 Oxidizing Agent

An oxidizing agent (also called an oxidant, oxidizer or oxidizer) can be defined as a substance that removes electrons from another reactant in a redox chemical reaction. The oxidizing agent is "reduced" by taking electrons onto itself and the reactant is "oxidized" by having its electrons taken away. Oxygen is the prime example of an oxidizing agent, but it is only one among many –

1. $KMnO_4$ (potassium permanganate).
2. $K_2Cr_2O_7$ (potassium dichromate).
3. O_3 (ozone).
4. F_2 (fluorine)

3.10.3 Potassium Permanganate:

1. Potassium permanganate is an inorganic chemical compound with the formula $KMnO_4$. It is a salt consisting of K^+ and MnO_4^- ions. Formerly known as permanganate of potash crystals, it is a strong oxidizing agent. It dissolves in water to give intensely purple solutions.

2. Potassium permanganate can be used to quantitatively determine the total oxidizable organic material in an aqueous sample. The value determined is known as the permanganate value. In analytical chemistry, a standardized aqueous solution of $KMnO_4$ is sometimes used as an oxidizing titrant for redox titrations (permanganometry).

3. For the standardization of $KMnO_4$ solutions, reduction by oxalic is often used.

Why $KMnO_4$ is stronger oxidizing agent compared to other oxidizing agents?

1. Because it contains Manganese in its highest oxidation state of +7.

2. This compound is a strong oxidizing agent because elements become more electronegative as the oxidation states of their atoms increase.

3. The permanganate in potassium permanganate has the anion MnO_4^- that is the reason for its strong oxidizing properties.

4. $KMnO_4$, being a very strong oxidizing agent, can react with a variety of groups.

5. Potassium permanganate oxidizes aldehydes to carboxylic acids, such as the conversion of *n*-heptanal to heptanoic acid:

$$5C_6H_{13}CHO + 2KMnO_4 + 3\,H_2SO_4 \rightarrow 5C_6H_{13}COOH + 3H_2O + K_2SO_4 + 2MnSO_4$$

6. Even an alkyl group (with a benzylic hydrogen) on an aromatic ring is oxidized, e.g., toluene to benzoic acid.

$$5C_6H_5CH_3 + 6KMnO_4 + 9H_2SO_4 \rightarrow 5C_6H_5COOH + 14H_2O + 2K_2SO_4 + 6MnSO_4$$

3.10.4 Potassium Dichromate

1. Potassium dichromate, $K_2Cr_2O_7$, is a common inorganic chemical reagent, most commonly used as an oxidizing agent in various laboratory and industrial applications. It is a crystalline ionic solid with a very bright, red-orange color. It is also known as potassium bichromate; bichromate of potash etc.

2. In organic chemistry, potassium dichromate is a mild oxidizer compared with potassium permanganate.

3. It is used to oxidize alcohols. It converts primary alcohols into aldehydes, or into carboxylic acids if heated under reflux.

4. In contrast, with permanganate, carboxylic acids are the sole products. Secondary alcohols are converted into ketones — no further oxidation is possible.

5. For example, menthone may be prepared by oxidation of menthol with acidified dichromate. Tertiary alcohols are not oxidized by potassium dichromate.

3.10.5 Comparison between $K_2Cr_2O_7$ and $KMnO_4$:

1. Both are powerful oxidants, but typically if one can use manganate you do so with preference over chromate. Chromate is dangerously toxic stuff.

2. Dichromate is +6 oxidation state, and this rapidly converts to chromate +5 which is one of the most powerful inorganic carcinogens. On the other hand, manganese is not that bad.

3. Unlikely to Potassium Permanganate, Potassium Dichromate is acutely and chronically harmful to health and must be handled and disposed of appropriately.

4. Potassium dichromate is carcinogenic and should be handled with gloves and appropriate health and safety protection; permanganate is not carcinogenic.

5. Unlikely to Potassium Permanganate, Potassium

6. Dichromate is corrosive and exposure may produce severe eye damage or blindness. Human exposure further encompasses impaired fertility, heritable genetic damage and harm to unborn children.

7. As an oxidizer, potassium permanganate is stronger than potassium dichromate.

3.10.6 Balancing Redox Equations

Balance the oxidation of Fe^{2+} to Fe^{3+} by $Cr_2O_7^{2-}$ in acid solution

1. Write the unbalanced equation for the reaction in ionic form.
$Fe^{2+} + Cr_2O_7^{2-} \rightarrow Fe^{3+} + Cr^{3+}$

2. Separate the equation into two half-reactions.
$Fe^{2+} \rightarrow Fe^{3+}$
$Cr_2O_7^{2-} \rightarrow 2Cr^{3+}$

3. Balance the atoms other than O and H in each half-reaction.

$Cr_2O_7^{2-} \rightarrow 2Cr^{3+}$

4. For reactions in acid, add H_2O to balance O atoms and H^+ to balance H atoms.

$Cr_2O_7^{2-} \rightarrow 2Cr^{3+} + 7H_2O$

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

5. Add electrons to one side of each half-reaction to balance the charges on the half-reaction.

$Fe^{2+} \rightarrow Fe^{3+} + 1e^-$

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

6. If necessary, equalize the number of electrons in the two half-reactions by multiplying the half-reactions by appropriate coefficients.

$6Fe^{2+} \rightarrow 6Fe^{3+} + 6e^-$

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

7. Add the two half-reactions together and balance the final equation by inspection. The number of electrons on both sides must cancel.

$6Fe^{2+} \rightarrow 6Fe^{3+} + 6e^-$

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

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

8. Verify that the number of atoms and the charges are balanced.

14x1 – 2 + 6x2 = 24 = 6x3 + 2x3

3.11 Experiment No. 7 Determination of Molar Concentration of Potassium Permanganate Using Oxalic acid

Theory: Potassium permanganate reacts with oxalic acid in presence of sulphuric acid as medium.

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 10CO_2$$

Chemicals:

Sulphuric acid solution (0.1M)
Potassium permanganate solution.
Oxalic acid solution (0.05M).

Method:

1. Clean the burette thoroughly, wash it with distilled water and finally rinse it with Potassium permanganate solution. Clamp the burette vertically in a burette stand.
2. Remove the funnel before noting initial reading of the burette.
3. Pipette 20ml of oxalic acid solution in conical flask.
4. Add 10ml of molar sulphuric acid solution.
5. Heat the conical flask contents to 60 to 70 C then titrate the contents using Potassium permanganate solution from burette; shake the flask gradually till the pink color appears.
6. Repeat the above steps and calculate the concentration of $KMnO_4$ in g/L.

Table 3.6: Titration of sodium hydroxide vs oxalic acid solution

No	V of $KMnO_4$	Burette readings		Concordant reading in ml
1		Initial reading (x)	Final reading (y)	
2				
3				

3.12 Experiment No. 8 Determination of Hydrogen Peroxide H_2O_2 Using $KMnO_4$ Solution (0.05N)

Theory: When we adding of potassium permanganate solution to hydrogen peroxide solution the permanganate solution color disappeared.

1. $MnO_4^- + 2H^+ \rightarrow Mn^{2+} + H_2O$

2. $H_2O_2 \rightarrow H_2O + O$
3. $2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow MnSO_4 + K_2SO_4 + 8H_2O + 10O$
4. $H_2O_2 + 2I^- + 2H^+ \rightarrow I2 + 2H_2O$

Chemicals: Potassium permanganate solution (0.05N), Sulphuric acid and Hydrogen peroxide solution (commercial)

Method:

1. Fill the 50ml capacity of pipette with the $KMnO_4$ solution above the mark. To obtain an accurate reading, you should have the calibration mark at eye level; i.e., your line of sight should be parallel with the mark.
2. Transfer the 10ml of the hydrogen peroxide solution H_2O_2 into the clean 250 ml conical flask.
3. Close the stopcock and wait a few seconds for drainage to be complete, note the reading on the burette to two decimal places ($V_{initial}$).
4. Titrate to the end point against standard solution of $KMnO_4$ solution from the burette to two decimal places (V_{final}).
5. Repeat the above process (three times).

Conclusion:

I. The concentration of H_2O_2 solution?
II. Calculate Strength of H_2O_2 solution in g/L?

3.13 Experiment No. 9 Determination of Sodium Nitrite using $KMnO_4$ Solution (0.05 N)

Theory: Sodium nitrite solution $NaNO_2$ reacts with potassium permanganate solution according to the following equation at 40. When potassium permanganate solution added from burette the nitrite solution lost some of nitroso acid don't react with permanganate for this, we add nitrite solution from burette instead of permanganate solution.

$$2KMnO_4 + 5NaNO_2 + 3H_2SO_4 \rightarrow MnSO_4 + K_2SO_4 + 5NaNO_3 + 3H_2O$$

Chemicals: Potassium permanganate solution (0.1N), Sulphuric acid (3N), Sodium nitrite solution $NaNO_2$

Method:

1. Fill the 50ml capacity of pipette with the Sodium nitrite solution $NaNO_2$ above the mark. To obtain an accurate reading, you should have the calibration mark at eye level; i.e., your line of sight should be parallel with the mark.
2. Transfer the 10ml of the potassium permanganate solution and 10ml of H_2SO_4 (3N) solution into the clean 250 ml conical flask the solution diluted with water add 150ml, and then heat at 40.
3. Close the stopcock and wait a few seconds for drainage to be complete, note the reading on the burette to two decimal places ($V_{initial}$).
4. Titrate to the end point against $NaNO_2$ solution from the burette to two decimal places (V_{final}).
5. Repeat the above process (three times).

Conclusion:

- The concentration of $NaNO_2$ solution?
- Calculate Strength of $NaNO_2$ solution in g/L?

3.14 Exp. No. 10 Determination of Percentage of Iron

The concentrations of redox-active species can be determined by redox titrations. In a redox titration, a measured sample of the unknown is titrated against a standard solution of a substance that will oxidize or reduce the unknown. In the present experiment you will take a sample containing iron, add acid to dissolve it (thereby converting all the iron to iron (II)), then use a solution containing permanganate ion, MnO_4^-, to oxidize this Fe^{2+} to Fe^{3+} ion. The percent of iron in the sample will be calculated from the amount of permanganate needed to oxidize fully all the Fe^{2+} ions. A solution of permanganate ion in sulfuric acid efficiently oxidizes Fe^{2+} to Fe^{3+}. The permanganate ion acts as its own indicator, as MnO_4^- is highly colored while Mn^{2+} is essentially colorless. The product of oxidation, the Fe^{3+} ion, is itself, slightly colored.

$$MnO_4^- + 5Fe^{2+} + 8\,H^+ \rightarrow Mn^{2+} + 5\,Fe^{3+} + 4H_2O$$

Self-indicators: $KMnO_4$ (purple) → Mn^{2+} (colorless)

Method:

I. Standardization of permanganate solution

Use distilled water at all times throughout the experiment.

1. Weigh three clean dry labeled 250 ml Erlenmeyer flasks on an analytical balance. Place about 0.135 grams of oxalic acid dihydrate, $H_2C_2O_4.2H_2O$, into each of the three separate flasks and reweigh the flasks containing the acid.
2. Set up a burette with $KMnO_4$ solution to be standardized by titration.
3. Dissolve each acid sample in about 25 ml of distilled water.
4. Take one flask and add 1-2 ml of concentrated sulfuric acid.
5. The solution to which the acid has been added should get quite warm, but, since the titration is to be done at elevated temperatures to prevent side reactions, this is desirable. Heat the solution further to 70; during the titration the solution should be kept between 60 and 80 .
6. Read the level in the permanganate burette (to hundredths of an ml) -- the initial reading -and then add the solution slowly from the burette into the flask with the warmed acid sample *with constant stirring.* The equivalence point is the first appearance of a pink color (excess MnO_4^-) that lasts, with stirring, for 30 seconds. When this is obtained, read the burette again -- the final reading.
7. Take a second flask with oxalic acid in it and add 1-2 **ml** of concentrated sulfuric acid. Repeat steps 7 and 8 above with this sample. Do a third trial with the third flask in the same manner.

I. Standardization

1. From the measured mass of the oxalic acid samples that you used, calculate the number of moles of oxalic acid in each case. Remember the oxalic acid was weighed out as a dihydrate.

2. Write a balanced oxidation-reduction equation for the reaction of oxalic acid with potassium permanganate in an acidic solution then, from the

indicated molar ratio, calculate how many moles of MnO_4^- must have been used in each of the three titrations. The products are carbon dioxide and manganese (II) ion.

3. With the calculated number of moles and the measured volume of solution used, calculate three values for the molarity of the permanganate solution. Report an average molarity.

II. Determination of iron

1. With the known molarity of the permanganate solution and the measured volumes used in the titration, calculate the number of moles of permanganate used in each of the trials.

2. Write a balanced oxidation-reduction equation for the reaction of iron (II) with permanganate in an acidic solution and, from the indicated molar ratio, calculate how many moles of iron were in each of your weighed out samples.

3. Calculate how many grams of iron were in each of your weighed out samples and then what mass percent of iron was present in each case. Report an average mass percent of iron in the unknown mixture.

Primary applications of $Cr_2O_7^{-2}$: Determination Fe^{2+} Indirect determination of oxidizing agents; A known excess of Fe^{2+} is added to the, sample which is oxidant such as MnO_4^- and the excess of Fe^{2+} is back titrated with $Cr_2O_7^{-2}$

Ethanol (C2H5OH)

1. $6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 6Fe^{3+} + 2Cr^{3+} + 7H_2O$

2. $3C_2H_5OH + 2Cr_2O_7^{2-} + 16H^+ \rightarrow 4Cr^{3+} + 3CH_3COOH + 11H_2O$

$$\frac{\text{moles of Fe}}{\text{moles of Cr 2023}} = \frac{\text{W Fe / Mwt of Fe}}{\text{V}} = \frac{6}{1}$$

3.15 Experiment No. 11 Determination of Sodium Oxalate using Potassium Permanganate

Permanganate solutions stain glassware and require stringent cleaning before and after use. If you see a brown material form, that is most likely MnO_2 which is hard to remove. Be sure to clean stopcocks and tips of burette and thoroughly rinse all glassware with water. Sulfuric acid (H_2SO_4) that contains a small amount of $MnSO_4$ as a catalyst is used to provide acid in the above reaction. In this lab experiment, you will perform titrations for an oxidation-reduction reaction (often called "redox" reaction) Potassium permanganate ($KMnO_4$) is a common chemical found in most laboratories. It is best known for its deep purple color that can be seen with the naked eye at low concentrations. It dissolves in water as a strong electrolyte to give K+ and MnO_4^- ions. Manganese is in the +7-oxidation state (the highest) in $KMnO_4$. Therefore, it can only be reduced (gain electrons) to lower oxidation states in redox reactions. Although it is possible to make +4, +3, 0 and other oxidation states, the most common reaction is a five-electron reduction to +2; that is Mn^{2+} which occurs as a hydrated ion in water. The reduction half reaction is:

$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$$

The half reaction requires acid (H^+) to make water, and the acid speeds up the reaction. There are many choices but one of the best is based on a chemical called sodium oxalate ($Na_2C_2O_4$). This compound has weak base properties, and in strong acid it is immediately converted to oxalic acid ($H_2C_2O_4$) but that's not what we are interested in for this experiment. The sodium form dissolves to give $2Na^+$ and one oxalate ($C_2O_4^{2-}$). The oxidation half reaction may then be written as:

$$C_2O_4^{2-} \rightarrow 2CO_2 + 2e^-$$

The two half reactions have different numbers of electrons, five for the reduction and two for the oxidation. If the first reaction is multiplied by 2 and the second by 5, then the two are added together we get the balanced, overall redox reaction:

$$5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \rightarrow 10CO_2 + 2Mn^{2+} + 8H_2O$$

This reaction tells us that five oxalate ($C_2O_4^{2-}$) and two permanganate (MnO_4^-) on a mole basis go together to make products in the presence of a lot of acid (H^+). A titration is a process of combining two liquids – a titrant and an analyte – in a manner so that stoichiometric equivalence is achieved. 2 mol permanganate = 5 mol oxalate. Suppose you make a solution that contains a known molarity of permanganate. Put that in a burette; deliver it into an oxalate solution until something happens that tells you that the reaction stoichiometry has been met. Then read the volume delivered and you may calculate the moles of oxalate from:

(5 mol oxalate /2 mol permanganate) x $M(MnO_4^-)$ x $V(MnO_4^-)$ = mol oxalate ($C_2O_4^{2-}$)

Method: Standardization of Prepared Permanganate Solution

1. Weigh 0.250 g of $Na_2C_2O_4$ into a 250 ml beaker or flask.
2. Dissolve this salt in about 100 ml of Distilled water, and acidify the solution with 25 ml of 3M H_2SO_4 (Be Careful!).
3. Fill the burette to the zero line and make sure there is no air bubble in the tip.
4. Titrate the solution with the prepared permanganate solution, remembering to stir constantly.
5. Stop the titration when the solution becomes slightly colored and the color lasts a minute or longer after thorough mixing.
6. Record the volume delivered to the nearest 0.01 ml.
7. If your solution turns a permanent muddy brown, this is an indication of MnO_2 formation and you should discard the entire trial and start again.
8. Calculate the mol $Na_2C_2O_4$ for each mass used from its molecular weight.
9. Calculate the molarity of the $KMnO_4$ titrant and record in your notebook and the data table below, Repeats steps 3 times.

	Trial 1	Trial 2	Trial 3
(Mass of $Na_2C_2O_4$ (g			
Moles of $Na_2C_2O_4$			
(Initial burette reading (ml			
(Final burette reading (ml			
(Total volume used (ml			
(Total volume used (l			
Molarity of KMnO4			
(Average molarity (M			

Part II. Analysis of an Unknown Oxalate

1. Weigh 0.750 g of the unknown solid into a 250 ml beaker.

2. Follow the same method (steps 3-8 above) as for the standardization to calculate the mass and percentage of $Na_2C_2O_4$ in the unknown sample.

3. Your instructor will check calculations and give you instructions on clean up and waste disposal.

3.16 Experiment No.12 Determination of Cu(II) using Titration with Sodium Thiosulfate Solution

In the previous unit, you have estimated the mount of ferrous iron, Fe^{2+}, in a sample of iron filings by using one redox titration, namely, permanganatometry. In this part, we would estimate the amount of copper in a given sample. Here too, you would perform experiments, of which is based on a redox reaction, iodometry.

The theory behind iodometric determination of cupric ions, Cu^{2+} is given along with the method details of the experiments. Reaction involving change of oxidation number or transfer of electrons among the reacting substances. The standard solutions are either oxidizing or reducing agents. The principal oxidizing agents are KMnO4, $K_2Cr_2O_7$, I_2, KIO_3, and $KBrO_3$. Frequently used reducing agents are Fe(II) and Sn(II) compounds, $Na_2S_2O_3$, As_2O_3, $Hg_2(NO_3)_2$, $CrCl_2$ or $CrSO_4$ and $TiCl_3$ or $Ti_2(SO_4)_3$.

Equations:

1. $2MnO_4^- + 5H_2C_2O_4 + 6H^+ \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2$
   (Pink) (Colorless)

2. $2MnO_4^- + 10Fe^{2+} + 16H^+ \rightarrow 2Mn^{2+} + 10Fe^{3+} + 8H_2$
   (Pink) (Colorless)

3. $Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$
   (Orange) (Green)

4. $2CuSO_4 + 4KI \rightarrow Cu_2I_2 + 2K_2SO_4 + I_2$
   (Blue) (White ppt)

5. $2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$
   (Sodium Thiosulphate) (Sodium Tetrathionate)

Objectives: After studying this part and performing the experiments, you will be able to:

1. define and differentiate between iodometry and iodirnetry,
2. explain the redox reactions involved in iodometry,
3. explain the use of indicator in iodometry and standardize the given sodium thiosulphate solution,
4. use the iodometric method in estimating Cu^{2+} ions,
5. To estimate the strength in g/L of a given copper sulphate solution being provided with an approx. N/30 sodium thiosulphate (hypo) solution.

Theory:

1. $2CuSO_4 + 4KI \rightarrow Cu_2I_2 + 2K_2SO_4 + I_2$
2. $2Cu^{2+} + 2I^- \rightarrow 2Cu^+ + I_2$
3. or $2I^-_{(aq)} \rightarrow I_2 + 2e-$
4. $2Cu^{2+} + 2e- \rightarrow 2Cu^+$

Iodometric Titrations: Iodine titrations in which some oxidizing agent liberate I_2 from an I^- solution and then liberated I_2 is titrated with a standard solution of reducing agent. Estimation of $CuSO_4$, $K_2Cr_2O_7$, $KMnO_4$, Fe^{3+}, H_2O_2.

Detection of the end point:

1. In this titration a solution of starch is used as indicator.
2. Starch reacts with iodine in the presence of iodide to form an intensely blue-colored complex, which is visible at very low concentrations of iodine.
3. The color sensitivity decreases with increasing temperature of the solution.
4. It is insoluble in cold water and inexpensive.
5. It gives a water-insoluble complex with iodine; the starch solution should not be added until just prior to the end point when the color begins to fade.

Preparation and Use of Starch Solution:

1. Make a paste of 0.1g of soluble starch with a little water, and pour the paste, with constant stirring, into 100 ml of boiling water, and boil for 1 minute. Allow the solution to cool and add 2-3 g of potassium iodide used freshly.
2. The same volume of starch solution should always be added in a titration.
3. In the titration of iodine, starch must not be added until just before the end point is reached.

The Starch-Iodine Complex

1. Numerous analytical methods are based on redox titrations involving iodine.
2. Starch is the indicator of choice for these methods because it forms an intense blue complex with iodine.
3. It responds specifically to the presence of I_2, not to a change in redox potential.
4. The active fraction of starch is amylose, a polymer of the sugar -D-glucose. Small molecules can fit into the center of the coiled, helical polymer.
5. A hydrolysis product of starch is glucose, which is a reducing agent.

Method

Pipette 10.0 mL of $CuSO_4$ solution into a 250 ml conical flask then add a few drops of dilute sodium carbonate solution until a faint permanent precipitate remains, and this is removed by means of a drop or two of acetic acid. Then 0.25 g KI is added and titrate the liberated iodine with standard solution of sodium. thiosulphate until the brown color of iodine fades, then add 0.4 mL of starch solution, and continue the addition of the thiosulphate solution until the blue colour commences to fade. Then add about 0.25 g of potassium thiocyanate or ammonium thiocyanate, preferably as a 10 per cent aqueous solution: the blue color will instantly become more intense. Complete the titration as quickly as possible. The precipitate possesses a pale pink color, and a distinct permanent end point is readily obtained

There are two reasons for such timing:

1. Is that the iodine–starch complex is only slowly dissociated, and a diffuse endpoint would result if a large amount of the iodine were adsorbed on the starch.
2. Is that most iodometric titrations are performed in strongly acid medium and the starch has a tendency to hydrolyze in acid solution.

Why KSCN or NH_4SCN is added?

When copper (II) is titrated iodometrically, the end point is diffuse unless thiocyanate ion is added. This is due adsorption of I_2 on the surface of the cuprous iodide precipitate and only slowly reacts with the thiosulfate titrant. The thiocyanate coats the precipitate with CuSCN and displaces the iodine from the surface. The potassium thiocyanate should be added near the end point since it is slowly oxidized by iodine to sulfate. The pH must be buffered to around 3. If it is too high, copper (II) hydrolyzes and cupric hydroxide will precipitate. If it is too low, air oxidation of iodide becomes appreciable because it is catalyzed in the presence of copper.

Calculations: Estimation of the strength of Copper (II) solution

1. Molarity of $Na_2S_2O_3$ solution = M3 – M2 .... mol dm^3

2. Volume of $Na_2S_2O_3$ solution used = V3 = ...ml
3. Volume of copper **(II)** solution taken ***V4*** = 20ml
4. Molarity of copper **(11)** solution **M4 -**?
5. Using molarity equation **M4 V4** = **M3 V3,**

3.17 Experiment No.13 Determination of Sodium Thiosulphate using Iodometric Method

Standardization of Sodium Thiosulphate Solution:

The standardization of thiosulphate solutions may be done with potassium iodate, potassium dichromate, copper and iodine as primary standards, or with potassium permanganate as secondary standards.

Iodimetric and iodometric titrations constitute another class of oxidation-reduction titrations where in either iodine solutions are employed directly for the assay or an equivalent amount of iodine is liberated indirectly from the reaction mixture and then assayed.

Iodimetry: is a method based on the following reversible reaction: $2I^- I_2 + 2e$, it can be utilized for the quantitative estimation of reducing agents like thiosulphates ($Na_2S_2O_3$) by employing a standard solution of iodine and it is an indirect procedure based on the a fore said reversible reaction where by the assay of oxidizing agents, for instance: available chlorine' in bleaching powder, cupric and ferric salts may be carried out by reducing them with an excess potassium iodide there by liberating an equivalent quantity of iodine which can be estimated using a standard solution of thiosulphate. Sodium thiosulphate ($Na_2S_2O_3.5H_2O$) is readily obtainable in a state of high purity, but there is always some uncertainty as to the exact water content because of the efflorescent nature of the salt and for other reasons. The substance is therefore unsuitable as a primary standard. It is a reducing agent by virtue of the half-cell reactions:

1. $2S_2O_3^{2-} \rightarrow S_4O_6^{2-}$
2. $I_2 + I^- \rightarrow I_3^-$

3. Starch + I_3^- Starch- I_3 (Starch-triiodide complex)
4. $2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I$

Preparation of 0.1N Iodine Solution

Iodine in aqueous solution acts as an oxidizing agent which forms the basis of assay methods involving direct titration with iodine. Thus, we have:

I_2+2e→2I–orI_2 ≡ 2e
Or 126.9g I_2 ≡ 1000ml nor 12.69g I_2 ≡ 1000ml0.1N
Or 3.17g I_2 ≡ 250ml 0.1N

Preparation of a 0.1N Sodium Thiosulfate Solution

Dissolve about 25g of sodium thiosulfate penta-hydrate crystals in 1liter of boiled distilled water. Add about 0.2g of sodium carbonate as a preservative. Shake to ensure complete dissolution and mixing. Avoid exposure to light, as this tends to decomposition.

Materials Required:

1. Iodine: 3.2g.
2. Potassium iodide: 7.5g.
3. sodium thiosulfate penta-hydrate

Method

Weigh accurately 3.2g of crushed iodine crystals on a watch glass and transfer to a beaker containing potassium iodide (7.5g) and water (10ml). Dissolve the contents of the beaker with the help of a glass rod and frequent swirling. Transfer the contents of the beaker quantitatively to a 250ml volumetric flask and make up the volume with distilled water.

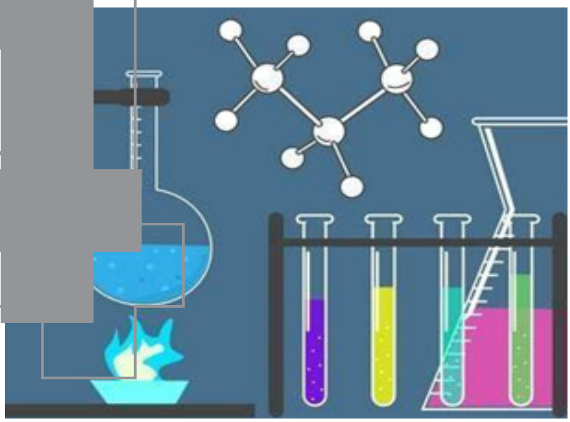
Explanation: Iodine is sparingly soluble in water but undergoes rapid dissolution in the presence of potassium iodide due to the formation of the corresponding triiodide I onto solubilized iodine in aqueous KI solution, and iniodometry act as reducing agent, the excess KI helps in retaining liberated I_2 in solution through interaction with KI.

Standardization of 0.1N Sodium Thiosulfate:

1. Weigh three pieces of clean copper wire or foil using about 0.20 to 0.25g each and place the minute's 250ml Erlenmeyer flasks.
2. Add to each flask 5ml of 6M nitric acid and dissolve the copper by warming the solution on a hot plate.
3. After the copper has dissolved, add 25mL of water and boil the solution for a few minutes.
4. Add 5ml of urea solution (1g in 20ml of water) and continue boiling for another minute.
5. Cool the solution and neutralize the excess acid with 4M ammonia in the hood. Use a dropper to add the ammonia carefully until a pale blue precipitate of copper (II) hydroxide is obtained. This is somewhat like a titration.
6. Add 5ml of glacial acetic acid to dissolve the precipitate. Cool the solution if it is warm.
7. To the first sample, add 3g of potassium iodide, cover the flask with a watch glass, and allow standing for 2min.
8. Then titrate with thiosulfate solution until the brownish color of iodine is almost gone or a light tan or "mustard ".
9. Add 5ml of starch solution and 2g of sodium thiocyanate.
10. Swirl the flask for about 15 seconds and complete the titration, adding thiosulfate drop wise.
11. At the end point the bluish-gray color of the solution disappears and the precipitate appears white, or slightly gray, when allowed to settle.
12. Treat the second and third samples in the same manner and titrate with thiosulfate.
13. Calculate the molarity of the thiosultate solution.

Chapter

4



Qualitative Analysis of Organic Compounds

Chapter Four

Qualitative Analysis of Organic Compounds

4. Qualitative Analysis of Organic Compounds

Today, organic compounds are key components of plastics, soaps, perfumes, sweeteners, fabrics, pharmaceuticals, and many other substances that we use every day. The value to us of organic compounds ensures that organic chemistry is an important discipline within the general field of chemistry. In this chapter, we discuss why the element carbon gives rise to a vast number and variety of compounds, how those compounds are classified, and the role of organic compounds in representative biological and industrial settings. The aim is to identify the given organic compound by the following qualitative tests.

Preliminary tests:

a) Physical state: Solid or Liquid.

b) Physical constant: Melting point for solids and Boiling Pint for liquids

Ignition test: Take 2 drops of the liquid or 20 mg of the solid in a nickel spatula and heat on the flame. If the compound burns with the sooty flame it is an aromatic compound (or) if it burns with a non-sooty flame the compound is aliphatic.

4.1 Exp. No. 1 Elemental Analysis in Organic Compounds

Table 4.1. Methods of functional group analysis of alcohols

No.	Methods	Observation	Results
1	Test for Nitrogen: To 2ml of the extract add a pinch of solid ferrous sulphate and heat it to boiling. Add carefully 3 or 4 drops of dil. H_2SO_4.	Prussian blue color is observed.	Nitrogen is present.
2	Test for Halogens: To 2ml of the extract add 4 drops of dil. nitric acid and heat to boil for 5 minutes. Cool and add few drops of silver nitrate.	White or pale-yellow precipitate	Indicates the presence of chloride/ bromide/ Iodide
	To the above ppt. Add diluted ammonia solution and shake.	Ppt. is soluble Ppt. is sparingly soluble Ppt. is insoluble	Is chloride Is bromide Is iodide
b.	To 5-6 drops of the extract add 1 ml of CCl_4 and one ml of $KMnO_4$ solution and shake well and one drop of acid	CCl_4 layer changes to orange brown color	Bromide is confirmed
c.	To 5-6 drops of the extract add 1 ml of CCl_4 and 1 ml of 20% sodium nitrite solution and shake well.	CCl_4 layer changes to purple color	CCl_4 layer changes to purple color
3.	Test for Sulphur: To 1 ml of the extract add 1 ml of freshly prepared sodium nitroprusside ($Na_2[Fe(CN)_5NO]\ 2H_2O$	Purple or violet red color is observed.	Sulphur is present

Solubility: Test the solubility of the compound separately in water, ether, 5% sodium bicarbonate solution, 5% sodium hydroxide solution, dilute HCl and concentrated Sulphuric acid.

Table 4.2: Methods solubility's tests of organic compound

No	H_2O	Ether	5% $NaHCO_3$	5% NaOH	Dil. HCl	H_2SO_4 Conc.	Class
1.	_	+	+	+	_		Strong acid (carboxylic acid)
2.	_	+	_	+	_		Weak acid (Phenol)

3.	_	+	_	_	+		Bases (amines)
4.							Neutral (aldehydes, ketones, amides, anilides, esters, ethers and nitro hydrocarbons)
	_	+	_	_	_	_	Neutral (Poly nuclear aromatic and halogen-ated hydrocarbons.)
5.	+	-	+	+	+	+	Miscellaneous (Carbohydrates Urea and thiourea)

Functional group analysis: Identify the functional groups present in the compound by qualitative tests. Wherever possible two confirmative tests should be carried out. The details are given in the table. Once the functional group is identified it has to be confirmed by preparing one or two derivatives (solid) and by determining their melting points.

Reporting: Report your observation and conclusions as below: Based on preliminary tests, extra elements, solubility, functional group analysis and derivatives the given compound is identified and confirmed as ---------------.

4.2 Experiment No. 2 Detection of elements other than Carbon and Hydrogen in organic compounds

Various fields of science rely on this type of analysis for quality control development, medicine, and engineering and change are just fields that utilize element analysis of organic compounds. The most common method of element analysis of organic compounds carbon, hydrogen, nitrogen, sulphur and oxygen analysis. Element analysis of compound is the process by which a sample is analyzed to determine elements present in what quality where is present of particular elements is determined.

4.2.1 Detection of Nitrogen and Sulphur together

This is the most dependable test for the detection of nitrogen, sulphur and halogens. This test is also known as sodium fusion test. In order to perform this test, first of all sodium extract or Lasaigne’s extract is prepared as described below:

4.2.2 **Method of preparation Lassaigne's extract**

Take a small piece of dry sodium in a fusion tube. Heat the tube slightly so that it melts to a shining globule. Add a pinch or the organic compound. Heat it slowly to start with so that the compound reacts with sodium metal. Now heat it strongly. Plunge the red-hot tube into a dish containing distilled water. Crush the contents with a glass rod and heat to boiling. Remove the insoluble matter by filtration. The filtrate is called Lassaigne's extract. Nitrogen sulphur and halogens present in an organic compound are detected by making use of lassaigne's extract. Detailed method follows.

4.2.3 Method of detection nitrogen

To small portion of lassaigne's extract (usually alkaline), add 2ml of freshly prepared ferrous sulphate solution and heat. Now add it 2-3 drops of ferrous chloride solution and acidify with concentrated hydrochloric acid. A Prussian blue color indicates the presence of nitrogen compound. If nitrogen present in the compound, the sodium extract would contain sodium cyanide NaCN formed during fusion. On adding the required reagents, sodium cyanide reacts to form ferric ferrocyanide which has Prussian blue color. The purpose of acidify the reaction mixture in the end is to dissolve any green participate of $Fe(OH)_2$ since it may lead to wrong internees'.

4.2.4 Detection of Nitrogen and Sulphat together

Organic compound contains nitrogen and sulphur sodium sulphocyanide (NaCNS) is formed during preparation of lassaigne's extract. Sodium sulphocyanide reacts with ferric chloride $FeCl_3$ and gives blood red coloration due to formation of ferric sulphocyanide. This color indicates to the presence of both nitrogen and sulphur in organic compounds.

4.2.5 Method of Detection Sulphur:

1. Sodium nitroprussside test: To small portion of lassaigne's extract add a few drops of nitro prusside solution. A purple color indicates the presence of sulphur.

2. Chemistry of test: During preparation of lassaigne's extract sulphur from the organic compound combines with sodium sulphide Na2S and it give purple color on reaction with sodium nitroprusside.

Lead acetate: Acidify a small portion of lassaigne's extract with acetic acid and add a few drops of lead acetate solution, the formation of black precipitate indicates the presence of sulphur in organic compound.

Silver nitrate test: To a small portion about 2ml of lassaigne's extract add 1ml of concentrated HNO_3 and boil for some time. Cool the contents and add to its silver nitrate solution.

a. White precipitate, soluble in ammonium hydroxide solution indicates the presence of chlorine in organic compound.

b. Pale yellow precipitate, sparing soluble in ammonium hydroxide it indicates the presence of bromine.

c. Yellow precipitate insoluble in ammonium hydroxide indicates the presence of iodine.

Chemistry of test: The function of adding concentration nitric acid and boiling is to decompose any sodium cyanide or sodium sulphide present in the reaction.

Carbon Disulphide test: Acidify a small portion of lassaigne's extract with diluted HCl and add a few drops of calcium disulphide or (CCl_4 or $CHCl_3$) now add freshly prepared chloride water shake vigorously appearance of orange color in the carbon disulphide layer

4.2.6 **Test for Alkenes and Alkynes**

Characteristic reactions differentiating saturated aliphatic hydrocarbons from unsaturated aliphatic hydrocarbons. Unsaturated hydrocarbons and other compounds with unsaturated bonds decolorize bromine solution in consequence of addition reaction. Any non–aromatic unsaturated hydrocarbon with a double or triple carbon–carbon bond. Bubble gas through, or add liquid to, a solution of bromine in hexane or water. The orange / brown bromine rapidly decolorizes, as a saturated colorless organic bromo–compound is formed.

4.2.7 The test of bromine addition

Unsaturated hydrocarbons and other compounds with unsaturated bonds decolorize bromine solution in consequence of addition reaction.

Reactions:

1. $R_2C{=}CR_2 + Br_2 \rightarrow BrR_2C{-}CR_2Br$
2. $RC{\equiv}CR + 2Br_2 \rightarrow Br_2RC{-}CRBr_2$

R = H, alkyl or aryl: Saturated alkanes give no fast reaction with bromine.

Method: Add dropwise 1 % acetic acid solution of bromine to about 0.5 ml of sample dissolved in acetic acid or other organic solvent. Mix carefully the contents of the test tube. The pink color of bromine quickly disappears to the moment, when total multiple bonds are saturated.

4.2.8 The test with potassium permanganate by Lehman

The solution of $KMnO_4$ submits decolorization in the presence of compounds with unsaturated bonds. This is the consequence of manganese reduction from +7 to +2. During reaction, the solution becomes alkaline (KOH is formed) and the brown precipitate of manganese dioxide can appear in the reaction mixture.

Method

Add dropwise 0.1 % acetone solution of $KMnO_4$ to about 1 ml of examined sample. Mix the solution carefully after addition of each drop and wait for disappearance of $KMnO_4$ pink color. The quantity of decolorized solution and the speed of its decolorizing depend on the amount of multiple bonds in the molecule of examined compound. The following methods of functional group detection.

Test for phenolic functional group:

(a) Neutral $FeCl_3$ test (*Functional group test*): Dissolve the given organic compound in water or alcohol and to this a drop or two of neutral solution. Appearance of red/green/pink/blue-violet colors confirms the presence of phenolic functional group.

Compound	Color with $FeCl_3$ solution
Phenol, O-cresol	Violet
p-cresol, quinol	Blue
m-cresol, naphthol (alcoholic)	Blue - violet
Resorcinol	Violet - blue
α- naphthol	Pink
β- naphthol	Green

Preparation of neutral $FeCl_3$ solution: Take the solution of $FeCl_3$ in a test tube and add NaOH solution till a small amount of precipitate is observed. Then add a drop or two of $FeCl_3$ solution to dissolve the precipitate.

(b) Phthalein test- The phenols having a *free para position* respond to this test. In a dry test tube, gently heat a small amount of given organic compound with an equal amount of phthalic anhydride (or phthalic acid) and conc. sulphuric acid (2-3 drops), for 1-2 minutes. Cool and pour the mixture into a beaker containing dilute sodium hydroxide solution. Appearance of pink, blue, green, red colorations indicate the presence of a phenol with free para position.

Compound	Color
Phenol, o-Cresol	Red
m-Cresol	Bluish purple
Catechol	Blue
Resorcinol	Red solution with green fluorescence
1-Naphthol	Green
2-Naphthol	Very faint green with slight fluorescence

4.2.9 Test for alcoholic functional group

1. The Lucas test (1 test per group).

Primary, secondary and tertiary alcohols react in different way with Lucas' reagent (the solution of anhydrous zinc chloride in concentrated hydrochloric acid). Primary alcohols, containing less than six carbon atoms, do not react with the reagent, and give clear, slightly dark solution. With secondary alcohols, turbidity of the solution appears, forming two phase solution after 1-1.5 hours. In the presence of tertiary alcohols, the solution turbidity and solution separation for two phases form very quickly. Tertiary alcohols react with concentrated HCl even in the absence of $ZnCl_2$.

Method

Add 5 ml of Lucas' reagent to three dry test tubes with ground-in stoppers, containing respectively primary, secondary and tertiary alcohols (0.5 ml). Mix the content for a while, put them away to the laboratory rack, and check the time difference, necessary in each case for turbidity and two-phase solution formation.

2. The test with mercury sulfate ($HgSO_4$) for tertiary alcohols by Deniges.

Only tertiary alcohols submit to this reaction. After dehydration, they form unsaturated hydrocarbons. In these conditions, secondary alcohols form only turbid solution and primary ones only opalescence, because of very low speed of alkenes formation.

Method

Add 1-2 drops of tertiary alcohol solution to about 1 ml of Deniges reagent (the solution of mercury sulfate in diluted sulfuric acid). Heat the sample in the boiling water bath until a yellow sediment appears.

3. Ceric ammonium nitrate test (*Functional group test*): Dissolve a small amount of given organic compound in minimum amount of water or dioxane (*for water insoluble compounds*) and add freshly prepared ceric ammonium nitrate solution (few drops. Appearance of red color shows the presence of alcoholic group.

4.Ceric ammonium nitrate test:

$$\underset{\text{Alcohol}}{2R{-}OH} + \underset{\text{Ceric ammonium nitrate}}{(NH_4)_2Ce(NO_3)_6} \longrightarrow \underset{\text{Pink or red complex}}{[(ROH)_2Ce(NO_3)_4]} + 2NH_4NO_3$$

5. Iodoform test: Iodoform test is given by alcohols which contain CH_3CHOHR group and oxidize to CH_3COR group during the reaction to give a positive iodoform test (same as described for carbonyl compounds).

When a compound containing CH_3CO- group or the group $CH_3CH(OH)$- (which can be easily oxidized to CH_3CO group), is treated with a solution of iodine in basic medium, iodoform, a yellow solid separated out.

Method

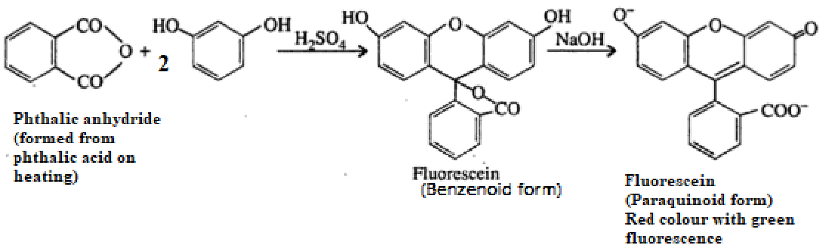
Dissolve a small amount of given organic compound in minimum amount of water and add sodium hydroxide (~1 mL, 10%). Add to this a saturated solution of iodine-potassium iodide in water with stirring until a dark color of iodine persists. Heat the solution in water bath and maintain the temperature at 80ºC for few minutes. Remove the color of excess iodine by adding a few drops of sodium hydroxide solution. A yellow precipitate of iodoform indicates the presence of CH_3CO group in the compound.

$$\underset{\text{2-Propanol}}{CH_3-CH(OH)-CH_3} + I_2 + 2NaOH \longrightarrow CH_3-\overset{O}{\overset{\|}{C}}-CH_3 + 2NaI + 2H_2$$

$$\underset{\text{Acetone}}{CH_3-\overset{O}{\overset{\|}{C}}-CH_3} + 3I_2 + 4NaOH \longrightarrow \underset{\text{Iodoform}}{CHI_3} + CH_3COONa + 3NaI + 3H_2O$$

Fluorescein Test:

Fluorescein Test



4.3 Experiment No. 3 Test for Alcohol R-OH Group

Alcohols are organic compounds that may be considered as derivatives of water in which one of the hydrogen atoms of substituted alkyl group. Therefore, properties of alcohols may be related to properties of both water and hydrocarbons. The alkyl group could be primary, secondary, or tertiary, and may be open chain or cyclic. Accordingly, alcohols may be defined as organic compounds that contain hydroxyl groups attached to alkyl, substituted alkyl, or cyclic alkyl group. Functional groups play a vital role in organic chemistry. The organic compound which has –OH functional group are called alcohols. Alcohols are compounds containing an –OH group bonded to tetrahedral carbon atom. The general formula of alcohol R-OH.

Physical properties

- Alcohols are colorless liquids with a special fain to dour. Benzyl alcohol and cyclo hexanol have characteristic odors.
- Aliphatic alcohols burn with blue flame (without smoke) while aromatic alcohols burn with yellow smoky flame.
- Boiling points of alcohols are considerably high (being as sociated liquids); they increase as the molecular weight (number of carbons) increases.
- Alcohols are miscible with water except benzyl alcohol, cyclo-hexanol, and *sec*-butanol (which is very slightly soluble in water.

Table 4.3: Functional group analysis of alcohols.

Chemical test	Methods	Observations
Hydroxy group R–OH		
in alcohols and phenols (in 'dry' conditions)	(1) Mix it with a few drops of ethanoyl chloride, test fumes with litmus and silver nitrate (note ethanoyl chloride reacts with water, phenols and amines). (2) Mix it with a little phosphorus (V) chloride and test as above. (3) Warm with a little ethanoic acid and a few drops of conc. sulphuric acid. Pour into water.	(1) Litmus turns red and a white precipitate with silver nitrate $_{(aq)}$ (drop on end of glass rod), if the mixture is poured into water, you may detect a 'pleasant' ester odor, can test for HCl but water and amines produce it too! (2) as for (1) but no ester smell! (3) You should get a 'pleasant' characteristic smell of an ester.

Primary alcohol		
RCH_2OH, R = H, alkyl or aryl (NOT a phenol). (2) is not a good test on its own, since so many other readily reducible organic compounds will give the same reaction, though following it up by testing for an aldehyde gives it much more validity.	(1) Luca's test – shake a few drops with cold zinc chloride in conc. $HCl_{(aq)}$ (2) Distil with potassium dichromate (VI) and mod. conc. $H_2SO_{4(aq)}$	(1) Solution remains clear. (2) If product distilled off immediately an aldehyde odor can be detected and the solution color changes from orange to green.
Secondary alcohol		
R_2CHOH, R = alkyl or aryl. (2) is not a good test on its own, since so many other reducible organic compounds will give the same reaction, though following it up by testing for a ketone gives it much more validity.	(1) Luca's test. (2) Distil with $K_2Cr_2O_7$/ $H_2SO_{4(aq)}$	(1) Solution may cloud very slowly or remains clear (hit and miss) (2) If product distilled off immediately a ketone odor can be detected and the solution color changes from orange to green. Chromium (III) ion. If the organic product is collected you could test for an aldehyde.
Tertiary alcohol		
R_3COH, R = alkyl or aryl.	(1) Luca's test. (2) Distil with $K_2Cr_2O_7$/ $H_2SO_{4(aq)}$	(1) Goes cloudy very quickly. (2) No aldehyde or ketone readily formed
Phenols		
Phenols (OH group is attached directly to aromatic ring) chemical test. R–OH, where R is aryl e.g., C_6H_5OH	Add a few drops of iron (III) chloride solution to a little of the phenol in water.	Usually gives a purple color. see also test for primary aromatic amines – use it in reverse starting with a known primary aromatic amine!).

Objective: To identify the presence of alcoholic functional group in a given organic compound.

Theory: Any of the following tests can be carried out to detect the alcoholic group. Sodium metal test, Ester test and Iodoform test.

4.3.1 Sodium metal test

General: It is based on the appearance of brisk effective vecence due to the libration of hydrogen gas when alcohol reacts with active metals such as sodium. The chemical reactions are given below:

1. $2R\text{-}OH + 2Na \quad R\text{-}ONa + H_2$
2. $2CH_3\text{-}OH + 2Na \quad CH_3\text{-}ONa + H_2$

The alcohol to be tested should be dry because water also reacts with sodium. Sodium should be handled carefully; un-reacted sodium should be destroyed by adding excess alcohol. This test is favorable is phenyl or carboxyl groups are absent.

Materials: Sodium metal. Acetic acid. Sodium hydroxide solution.

Apparatus: Test tubes, Holder, Filter

Methods

Take the organic compound to be tested in a dry test tube. Add 1g of anhydrous calcium sulphate and shake well to remove excess water. Decant the solution to another clean test tube. Add a small piece of sodium metal. If brisk effer vescence appears due to the evolution of hydrogen gas indicates the presence of alcoholic group**.**

1. $6C_6H_5OH + FeCl_3 \rightarrow H_3[Fe(OC_6H_5)_6] + 3HCl$

Ceric ammonium nitrate test (*Functional group test*): Dissolve a small amount of given organic compound in minimum amount of water or dioxane (*for water insoluble compounds*) and add freshly prepared ceric ammonium nitrate solution (few drops. Appearance of red color shows the presence of alcoholic group.

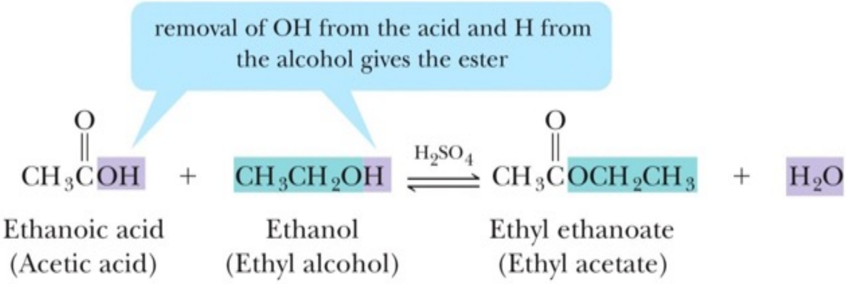
2. $ROH + (NH_4)Ce(NO_3)_6 \rightarrow [(ROH)_2Ce(NO_3)_4] + 2NH_4NO_3$

4.3.2 Ester test

Esters are found in fats and oils responsible for the aroma and flavor of bananas, oranges and strawberries. Esters can be prepared by treating a carboxylic acid with an alcohol in the presence of an acid catalyst, this reaction is slow reaction catalyzed by concentrated sulphuric acid.

Commonly H_2SO_4 or gaseous HCl. The main chain of an ester comes from the carboxylic acid, while the alkyl group in an ester comes from the alcohol. Esters give pleasant fragrances and flavors to many fruits and flowers.

Reactions:



A sweet smell indicates the presence of alcoholic group.

Materials: Glacial Acetic acid, Ethanol, methanol Concentrated H_2SO_4 and Sodium bicarbonate solution.

Apparatus: Beakers, Measuring cylinder.

Methods:

Take 1ml of organic liquid to be tested in clean test tube. Add 1ml of glacial acetic acid and 2-3 drops of concentrated sulphuric acid. The hot mixture is poured into a beaker containing cold water. Smell the water in beaker. Fruity smell confirms the presence of alcoholic groups.

4.3.3 Acetyl chloride test

Alcohol reacts with acetyl chloride results in formation of ester and hydrogen chloride. The resulting hydrogen chloride HCl on contact with ammonium chloride and water.

1. R–OH + CH_3COCl → CH_3COOR + HCl an ester and hydrogen chloride are formed
2. R–OH + PCl_5 → R–Cl + $POCl_3$ + HCl

Chloro compound and hydrogen chloride are formed. From the hydrogen chloride fumes dissolved in water.

3. $Ag^{+}_{(aq)} + Cl^{-}(aq) \rightarrow AgCl_{(s)}$
4. $CH_3CH_2OH + RCOCl \rightarrow RCOOCH_2CH_3 + HCl$
5. $HCl + NH_4OH \rightarrow NH_4Cl + H_2O$
6. $R_3COH + HCl \rightarrow R_3CCl + H_2O$

Materials: Anhydrous Calcium sulphate. Organic compounds such as: Ethanol, methanol etc. Acetyl chloride and Ammonium hydroxide.

Apparatus: Test tubes, Measuring cylinder.

Method

Take 2ml of given organic compound in dry test tube. Add 1g of anhydrous calcium sulphate and shake well. Filter the solution. To the filtrate add 3 to 4 drops of acetyl chloride shake well. Take a glass rod dipped in ammonium hydroxide solution. Drying the glass rod near the mouth of the test tube. If white fume occurs then the presence of alcoholic group.

4.3.4 Iodoform test

Iodoform test is given by alcohols which contain CH_3CHOHR group and oxidize to CH_3COR group during the reaction to give a positive iodoform test (same as described for carbonyl compounds). When a compound containing CH_3CO- group or the group $CH_3CH(OH)$- (which can be easily oxidized to CH_3CO), is treated with a solution of iodine in basic medium, iodoform, a yellow solid separate out. This test given by secondary alcohols. Reactions and acetaldehyde. First the compound is heated with NaOH solution and iodine. A formation of yellow precipitate of iodoform shows the presence of alcohol, the chemistry of reactions given below.

1. $CH_3\text{-}CH(OH)\text{-}CH_3 + I_2 + 2NaOH \rightarrow CH_3\text{-}CO\text{-}CH_3 + 2NaI + 2H_2O$

We will do the iodoform test only twice with known alcohols, once with an alcohol that contains the methyl secondary alcohol functionality and once with a compound that does not. You will need two medium sized test tubes. Make sure there is no acetone present in your test tubes. Acetone also gives a positive Iodoform Test.

1. CH_3-CO-CH_3 + 3I_2 + 4NaOH → **CHI_3** + CH_3COONa + 3NaI + 3H_2O

Materials: Organic compounds such as: Ethanol, methanol. Iodine solution 1%. Sodium hydroxide.

Apparatus: Test tubes, Measuring cylinder and Water bath.

Method

Dissolve a small amount of given organic compound (1 ml) in minimum amount of water and add sodium hydroxide (1 ml, 10%). Add to this a saturated solution of iodine-potassium iodide in water with stirring until a dark color of iodine persists. Heat the solution in water bath and maintain the temperature at 80ºC for few minutes. Remove the color of excess iodine by adding a few drops of sodium hydroxide solution. A yellow precipitate of iodoform indicates the presence of CH_3CO group in the compound.

The mechanism: of the reaction is somewhat complex and will be studied in detail in the second semester. Only the outline of the mechanism is shown here. Notice that the first step is the formation of the mild oxidizing agent sodium hypoiodite, NaOI, from sodium hydroxide and iodine. NaOI oxidizes the alcohol to a carbonyl in the second step. NaOI is similar to sodium hypochlorite, NaOCl, which is the active ingredient in household bleach. Notice that the oxidation state of the halogen is +1, not the usual –1. In the second step, the alcohol is oxidized to a ketone (loss of two hydrogens) and the oxidation state of the iodine changes from +1 to –1 (gain of 2 electrons). In the third step, which really consists of several individual steps, the methyl ketone is converted to the anion of a carboxylic acid (with one carbon less than the alcohol) and iodoform (yellow precipitate). Only the second and third steps involve oxidation/reduction. **Note that** the initial product formed is a methyl ketone derivative and acetone contain this functionality. Acetone therefore gives a positive Iodoform Test. Be sure that your test tube does not contain any traces of acetone.

1. I – I +Na^+OH^- → Na^+I^- + IO^-Na^+
2. R-CH(OH)-CH_3 + IO^-Na^+ → R-CO-CH_3 + H_2O + I^-
3. CH3-CO-CH_3 + 3I_2 → R-CO-O- +CHI_3 + 3H_2O + 3I^-

4.3.5 Distinction between (1°), (2°), (3°) alcohols

In this experiment you are going to do a series of tests in order to determine whether or not an alcohol is a primary (1°), secondary (2°) or tertiary (3°) alcohol. The tests can also determine whether or not there is secondary methyl alcohol functionality in the molecule. You will do four chemical tests:

1. Chromic Acid Test (or Jones Oxidation).
2. Ritter Test using potassium permanganate.
3. The Lucas Test using $ZnCl_2$ and HCl.
4. The Iodoform Test.

4.4 Experiment No. 4 Reaction with acidified potassium dichromate solution

Acidified potassium dichromate solution changes from orange to green when a primary or secondary alcohol is added to it. There is no reaction between acidified potassium dichromate solution and tertiary alcohol. The orange $Cr_2O_7^{2-}{}_{(aq)}$ ions are reduced to green $Cr^{3+}{}_{(aq)}$ ions as the alcohol is oxidized. Acidified potassium dichromate solution changes from orange to green when ethanol is added to it. Not usually reactive enough to form a primary halogenoalkane

1. $R–CH_2OH + [O] \rightarrow R–CHO + H_2O$ or the full works!
2. $3R–CH_2OH + Cr_2O_7^{2-} + 8H^+ \rightarrow 2Cr^{3+} + 3R–CHO + 7H_2O$

The orange dichromate (VI) ion is reduced to the green chromium (III) ion. If the organic product is collected you could test for an aldehyde.

May be reactive enough to slowly form an insoluble secondary halogenoalkane.

1. $R_2CHOH + HCl \rightarrow R_2CHCl + H_2O$
2. $R_2CHOH + [O] \rightarrow R–CO–R + H_2O$ or the full works!
3. $3R_2CHOH + Cr_2O_7^{2-} + 8H \rightarrow 2Cr^{3+} + 3R–CO–R + 7H_2O$

The orange dichromate (VI) ion is reduced to the green. Reactive enough to immediately form an **insoluble** tertiary halogenoalkane**.** Stable to modest oxidation.

4.4.1 Luca's test

This test distinguishes tertiary, secondary and primary alcohols from each other. It uses zinc chloride as the reagent in concentrated hydrochloric acid (Lucas Reagent). It is based on the rate of formation of insoluble alkyl chloride. An emulsion is formed. This test is reliable only for alcohols that are fairly soluble in water.

1. Tertiary Alcohols React immediately to form an emulsion of the alkyl halide and water (cloudy solution).
2. Secondary Alcohols React in 5-10 minutes. Heating in warm water and shaking is sometimes necessary with water-insoluble alcohols.
3. Primary alcohols these generally take more than one hour to react.

The mixture of zinc chloride and concentrated hydrochloric acid is called Luca's reagent, it reacts with primary, secondary and tertiary alcohols at different rates. This reagent forms a colorination on reacting with alcohols. Tertiary alcohols react immediately and give cloudiness, secondary alcohols react slowly and gives cloudiness after five to ten minutes and there is no reaction with primary alcohols.

1. $CH_3CH_2OH + ZnCl_2 + HCl \rightarrow$ No reaction
2. $CH_3CHOHCH_3 + ZnCl_2 + HCl \rightarrow CH_3CHCl\text{-}CH_2CH_3$
3. $(CH_3)_3C\text{-}OH + ZnCl_2 + HCl \rightarrow (CH_3)_3C\text{-}Cl$
4. $ZnCl_2 + HCl \rightarrow ZnCl_3^- + H^+$

Benzyl alcohol shows the fastest positive result. Tertiary alcohols are faster in the formation of conjugated halides than secondary alcohols. Primary alcohols and methanol don't react and don't form two layers.

Method

Set-up three small test tubes as above, labeling each of them. Add 0.5 mL of the test alcohol to each test tube. To the first test tube, add 3 mL of the Lucas reagent. Shake vigorously using a small cork to stopper the test tube. Wait at least 10 minutes. If you have a water insoluble alcohol (you see two layers in your test tube) then repeat the experiment using a hot water bath set at 60 °C, immersing the test tube in the water bath immediately after shaking and waiting the 10 minutes with occasional shaking. Also repeat the experiment using a water-soluble alcohol of the appropriate class. Record your results. Repeat on all three classes of alcohol.

Method: Mix 2-4 drops of the alcohol with few drops of Lucas reagent and observe the results:

a. benzyl alcohol gives immediate result as shown by the appearance of two phases.
b. tertiary alcohols give two phases that separate within 2-3 minutes.
c. secondary alcohols give two phases that separate after 15-20 minutes (giving a cloudy solution).
d. in primary alcohols one layer appears.

4.4.2 Chromic Acid Oxidation of Alcohols

The experiment has three parts, all of which can be done in one laboratory session. First, you will practice all four of the chemical tests using known alcohols. You will perform each of the first three tests three times, once with a primary, once with a secondary and once with a tertiary alcohol. The fourth test we will do two times, once with a secondary methyl containing alcohol and once with a not secondary methyl alcohol.

Method

Set-up three small test tubes in your test tube rack. The tubes do not need to be dry. Label the first test tube as a primary alcohol, the next as a secondary alcohol and the third as a tertiary alcohol. Write down in your notebook which alcohols you are going to be using. Add 2 ml of acetone to each test tube and then add 3-4 drops of your test alcohol. Be sure that the drops fall into the acetone and do not remain on the sides of the tube. Add 2 drops of the Chromic Acid Test Reagent (also called the Bord well-Wellman Reagent). Shake vigorously using a small, tight-fitting cork. You should see a color change to a blue or blue-green or similarly colored precipitate within a few seconds to indicate a positive test. Record your results in your notebook.

4.4.3 Baeyer's test using potassium permanganate

This test is similar to the Chromic Acid Oxidation and provides the same information. It is the oxidation of primary and secondary alcohols to carboxylic acids and ketones using potassium permanganate ($KMnO_4$). Again, tertiary alcohols cannot be oxidized by this reagent because there is no hydrogen to be lost from the carbon that bears the OH group. In the Ritter Test the Mn^{7+} of $KMnO_4$ (bright purple) is reduced to Mn^{2+}. The Mn4+ is brownish in color. The reactions involved are as follows.

Method

As for the Chromic Acid Oxidation, set-up three small, labeled tests tubes. Add 2ml acetic acid to each tube. Add 3-4 drops of the test alcohol to each tube and then add *One* drop of saturated $KMnO_4$ solution to each test tube. Shake vigorously to mix, using a small cork. For the 1° and 2° alcohols you should see a brownish color develop as the purple $KMnO_4$ color disappears. Do not add too much $KMnO_4$. If you add an excess of this reagent, the purple color will persist even though you have a primary or secondary alcohol. With the tertiary alcohol you should see no color change since the purple color remains.

4.5 Exp. No. 5 Test for Carbonyl Compounds group (C=O)

Aldehydes and ketones constituents an important class of organic compounds containing the carbonyl functional group. Aldehydes has the structure RCH(O), while the ketones have the structure $R_2C(O)$, where R may be an alkyl or aryl. (R–CHO, R = H, alkyl or aryl) to distinguish from ketones ($R_2C=O$, R = alkyl or aryl) and also reducing sugars.

Objective: To identify the presence of aldehydes or ketones functional group in given organic compounds.

Theory: Aldehydes and ketones of low molecular weights are volatile compounds. Identification of aldehydes and ketones is based on two types of reactions addition reaction to the double bond and oxidation reaction.

4.5.1 Reaction with sodium bis-ulphite

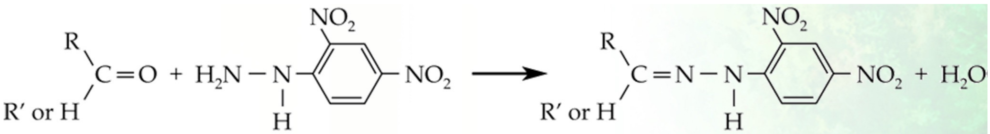
Take a saturated solution of sodium bisulphite in a clan test tube. Add 1 ml of the given organic compound to be tested. Shake well and leave for 15 to 20 minutes. If there is a formation of the white precipitate then the presence of the carbonyl group is confirmed.

R-CHO + $NaHSO_4$ → R_2-CO + $NaHSO_4$

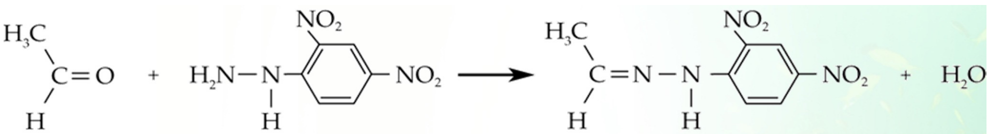
In aldehyde the carbonyl attached to a hydrogen atom and aliphatic or aromatic group. Formaldehyde is an exceptional case in which the carbonyl presents in formular attached to two hydrogen atoms. In ketones the carbonyl group attached to two aliphatic or aromatic groups.

4.5.2 Reaction with 2,4-dinitrophenylhydrazine

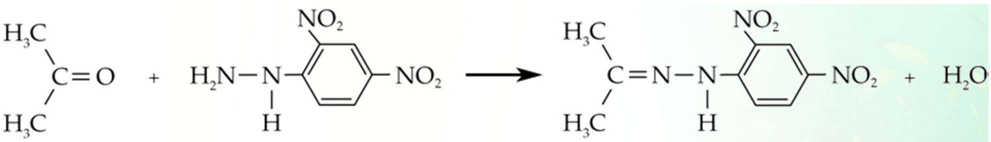
reacts with aldehyde or ketone to give yellow, orange or red precipitate of **2,4-dinitrophenylhydrazone**. This reaction is used to test for the presence of carbonyl group (C=O). Ethanal 2,4-dinitrophenylhydrazine ethanal 2,4 dinitrophenylhydrazone yellow precipitate



2,4-dinitrophenylhydrazone yellow precipitate



2,4 dinitrophenylhydrazone orange-yellow precipitate



The aldehyde or ketone with 2,4–dinitrophenylhydrazone is formed

$R_2C=O + (NO_2)_2C_6H_3NHNH_2$==>$(NO_2)2C_6H_3NHN=CR_2 + H_2O$

(R = H, alkyl or aryl): This tells you it's an aldehyde or ketone, but can't distinguish them, Aldehydes are stronger reducing agents than ketones and reduce the metal ion and are oxidized in the process.

Table 4.4: Functional group analysis of carbonyl compounds.

Chemical test	Methods	Observations
Aldehydes chemical test		
(R–CHO, R = H, alkyl or aryl) to distinguish from ketones ($R_2C=O$, R = alkyl or aryl) and also reducing sugars. Note (1) Test (b)(1) and (2) can be used to distinguish aldehydes (reaction) and ketones (no reaction). (2) Aromatic aldehydes do Not give a positive result with (b)(2) Benedict's or Fehling's reagent). (3) Reducing sugars may also give a positive test with (b)(1)/(2) reagent e.g. glucose (aldohexose) but not fructose? (ketohexose)?	(a) Add a few drops of the suspected carbonyl compound to Brady's reagent (2,4–dinitrophenylhydrazine solution)	(a) A yellow–orange precipitate forms with both types of carbonyl compound.
	(b) (1) warm a few drops of the compound with **Tollens' reagent** (ammoniacal silver nitrate) **(b)(2)** simmer with **Fehling's** or **Benedict's** solution (a blue complex of $Cu^{2+}_{(aq)}$)	**(b)** Only the aldehyde produces **(1)** A silver mirror on the side of the test tube. **(2)** A brown or brick red ppt.

There are generally distinguished by the following tests: Schiff,s test, Fehling test, Tollen's test and Chromic acid test.

4.5.3 Schiff's test: Schiff's reagent is prepared by passing sulphur dioxide into a solution of the dye fuchsine. The solution becomes colorless due to the formation of an additional product. Aldehydes abstract sulphurous acid from Schiff's reagent are restore the pink color, ketones in general do not respond to this reaction.

4.5.4 Fehling's Test: Fehling's solution is complex compound of Cu^{2+} when the aldehyde is treated with Fehling, s solution Cu^{2+} is reduced to Cu^{+} and the aldehyde is reduced to acids. During the reaction precipitate formed. Reduction of silver (I) ion to silver metal.

1. $RCHO + [O] \rightarrow RCOOH$,
2. $RCHO + 2Ag^{+} + H_2O \rightarrow RCOOH + 2Ag + 2H^{+}$

Reduction of copper (II) to copper (I) i.e., the blue solution of the Cu^{2+} complex changes to the brown/brick red color of insoluble copper (I) oxide Cu_2O.

3. $RCHO + 2Cu^{2+} + 2H_2O \rightarrow RCOOH + Cu_2O + 4H^{+}$

Method

Fehling, s solution is prepared by mixing equal amounts of Fehling A and Fehling B solutions. Take the given organic compound in a clean dry test tube. Add Fehling solution to it and heat the solution gently. If a brick red precipitate appears then the presence of aldehydes confirmed.

4.5.5 Tollens' reagent reaction

Tollens' reagent is used to distinguish between an aldehyde and a ketone. It is a specific test for aldehydes as Tollens' reagent has no reaction with ketones. Tollens' reagent is an aqueous solution of silver nitrate in excess ammonia. It contains the ***di-ammine-silver(I) ion***, $[Ag(NH_3)_2]^{+}$, in an alkaline solution. When an aldehyde reacts with Tollens' reagent, a **silver mirror** forms inside the test tube. Practically, it is possible to coat silver metal (like a mirror) on the inner wall of the test tube. However, grey or black precipitate forms if the inner wall of the test tube is not clean enough. During the reaction, diammine silver (I) ion is reduced to silver by the aldehyde.

1. $RCHO + 2[Ag(NH_3)_2]^+ + 3OH^- \rightarrow RCOO^- + 2Ag + 4 NH_3 + 2H_2O$
2. $CH_3CHO + 2[Ag(NH_3)_2]^+ + 3OH^- \rightarrow CH_3COO^- + 2Ag + 4 NH_3 + 2H_2O$

Method

Take 1 ml of silver nitrate solution in a clean test tube. Add dilute sodium hydroxide solution to it, and a brown precipitate form. Add dilute ammonia solution drop wise till the brown precipitate of Ag_2O dissolves. To this freshly prepared Tollens, reagent, add the given organic compound to be tested. Place the test tube in warm water. If there is appearance of silver mirror on the sides of the test tube confirms the presence of aldehydes.

4.5.6 Chromic acid test

Aldehydes react with chromic acid and gives a green to blue precipitate, ketones do not react with chromic acid.

1. $R\text{-}CHO + 2CrO_3 + 3H_2SO_4 \rightarrow 3R\text{-}C(O)\text{-}OH + 3H_2O + Cr_2(SO_4)_3$

Method

Take the given organic compound in a clean dry test tube. Add 1 ml of chromic acid reagent to the given organic compound. The appearance of a green or blue color precipitate indicates the presence of aldehyde.

Table 4.5: Formation of Iodoform test for Methyl ketone

No.	Methods	Observation	Results
1	To 3 drops of compound add 10 mL of 20% aq NaOH and shake well add gradually with shaking kI+I2 solution till dark color of iodine persist. Now warm the solution on a hot water bath for 10min.	A pale-yellow ppt. (iodoform) is formed. If no ppt. is formed after prolonged heating.	Methyl ketone is confirmed. The ketone is not methyl ketone.
2	To 0.5ml of ketone shake well. add carefully 3ml of 10% KI solution and 10ml of freshly prepared NaOCl and shake vigorously.	A pale-yellow ppt. (iodoform) is formed	Methyl ketone is Confirmed.

4.6 Experiment No. 6 Test for Carboxyl group (−COOH)

Physical Properties of Carboxylic Acids

In the liquid and solid states, carboxylic acids are associated by hydrogen bonding into dimeric structures. They are polar compounds and form very strong intermolecular hydrogen bonds. Carboxylic acids are more soluble in water than are alcohols, ethers, aldehydes, and ketones of comparable molecular weight. The lower carboxylic acids are freely miscible with water due to the presence of intermolecular hydrogen bonding with H_2O molecules. However, the solubility in water decreases gradually due to increase in the size of alkyl group. Aliphatic carboxylic acids up to nine carbon atoms are colorless liquids at room temperature with unpleasant odors. The higher acids are wax like solids.

Solubility in Water: Carboxylic acids form hydrogen bonds with many water molecules with one to four carbon atoms are very soluble in water as the number of carbons increases; the solubility of the carboxylic acid in water is reduced.

1. Test for carboxylic acid group

(a) Litmu's test- Add blue litmus solution (1 drop) to an aqueous solution of acid, appearance of a red color indicates the presence of a carboxylic acid (blue litmus paper may be used in place of a blue litmus solution).

(b) Sodium bicarbonate test (*Functional group test*): To a saturated solution of sodium bicarbonate add small amount of given organic compound. Brisk effervescence indicates the presence of carboxylic acid group.

(c) Fluorescein test: Heat a small amount of organic compound, resorcinol and conc. sulphuric acid (1-2 drops) in a clean and dry test tube till a dark brown colored liquid is formed. Then add few drops of this solution into a beaker containing dilute NaOH solution (10 mL NaOH diluted to 100 mL). Appearance of red color solution with green fluorescence indicates the presence of dicarboxylic acid.

(d) Test for Oxalic acid (Blue ring test): Heat a small amount of given organic compound, resorcinol (2-3 flakes) and water (1 mL) in a test tube. Cool the contents and add few mL of conc. H_2SO_4 along the sides of the test tube. Appearance of blue ring at the junction of two layers confirms the presence of oxalic acid.

Table 4.6: Methods Carboxylic Acids Functional Group Analysis.

No.	Methods	Observation	Results
1	Solubility in sodium $NaHCO_3$: To 50mg of the compound add 1ml of 10% sodium bicarbonate solution. To the clear solution add a few drops of conc. HCl.	The compound dissolves with effervescence. The compound is regenerated.	Presence of COOH group Compound is a carboxylic acid.
2	Esterification: To 50mg of the compound add 1 ml of ethyl alcohol or methanol and add 1Ml of conc. H_2SO_4 and heat the mixture in hot water bath for 5mts. Pour the contents into cold 10% sodium bicarbonate solution.	Fruity odor is Observed.	Compound is a carboxylic acid
	Ferric chloride coloration: To 50 mg of the compound add few drops of ammonium hydroxide until alkaline to litmus. Add 1 ml of neutral Ferric chloride solution.	A brownish violet or reddish brown ppt is formed.	Carboxylic acid is confirmed

4.6.1 Sodium bicarbonate test: (Functional group test)

To a saturated solution of sodium bicarbonate add small amount of given organic compound. Brisk effervescence indicates the presence of carboxylic acid group. Reaction with sodium carbonate/sodium hydrogen carbonates solution. When a carboxylic acid reacts with sodium hydrogen carbonate or sodium carbonate solution to form carbon dioxide which turns limewater milky.

$$CH_3COOH + Na^+HCO_3^- \xrightarrow{H_2O} CH_3COO^-Na^+ + H_2CO_3$$

$$H_2CO_3 \longrightarrow CO_2 + H_2O$$

$$CH_3COOH + Na^+HCO_3^- \longrightarrow CH_3COO^-Na^+ + CO_2 + H_2O$$

Sodium hydrogen carbonate

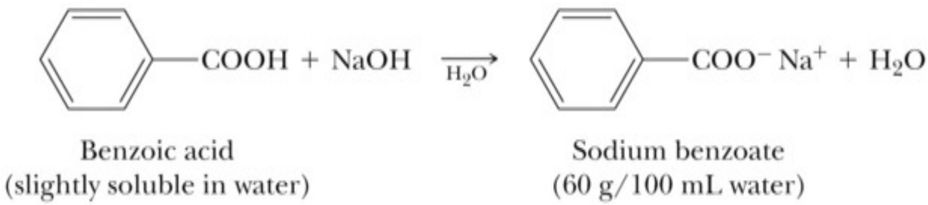


Table 4.7: Formation of Carboxylic acids salts

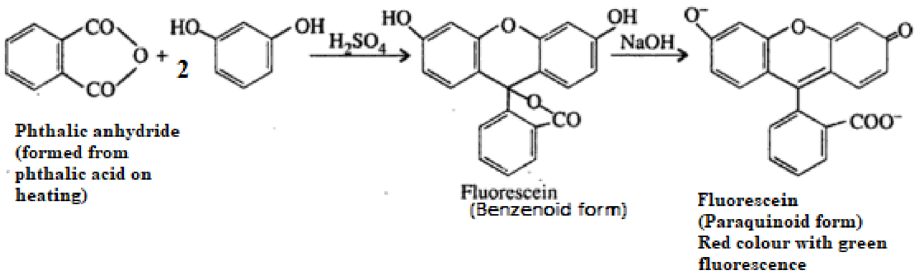
Carboxylic acids RCOOH	Mix the carboxylic acid with water and add a little sodium hydrogen carbonate solid or solution.	fizzing, colorless gas gives white precipitate with limewater
Salts of aliphatic carboxylic acids e.g., $RCOO^-Na^+$ or $(RCOO^-)_2Mg$ etc.	Add a little dilute hydrochloric/sulfuric acid to a suspected salt of an aliphatic carboxylic acid.	The solid or solution should have no strong odor, but after adding the mineral acid you should get a pungent odor of the original aliphatic acid. If it's the salt of an aromatic carboxylic acid, you get little odor and maybe a white crystalline precipitate.

The stronger acid, HCl/H_2SO_4 displaces the weaker aliphatic carboxylic acid which have strong–pungent characteristic odors e.g., ethanoic acid from an ethanoate salt (smell of acetic acid, vinegar) and butanoates release butanoic acid (butyric acid, rancid odor).

4.6.2 Fluorescein test:

Heat a small amount of organic compound, resorcinol and conc. sulphuric acid (1-2 drops) in a clean and dry test tube till a dark brown colored liquid is formed. Then add few drops of this solution into a beaker containing dilute NaOH solution (10 ml NaOH diluted to 100 ml). Appearance of red color solution with green fluorescence indicates the presence of dicarboxylic acid.

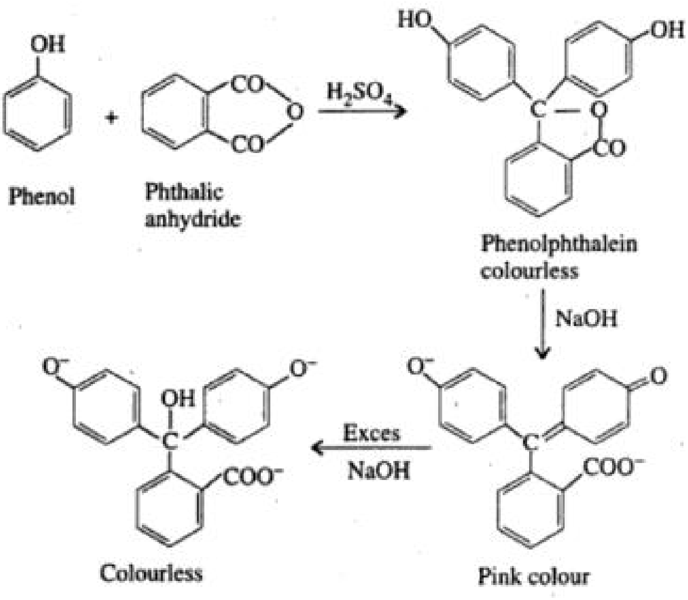
Fluorescein Test



Test for Phenolic functional group: Ferric chloride test: (Taking phenol as an example).

$6C_6C_5OH + FeCl_3 \rightarrow H_3[Fe(OC6H_5)_6] + 3HCl$

Phthalein test: (Taking example of phenol)



4.6.3 Test for Oxalic acid (Blue ring test)

Heat a small amount of given organic compound, resorcinol (2-3 flakes) and water (1ml) in a test tube. Cool the contents and add few ml of conc. H_2SO_4 along the sides of the test tube. Appearance of blue ring at the junction of two layers confirms the presence of oxalic acid.

4.6.4 Lactic acid (α-hydroxypropionic acid) detection

Organic acids with α-hydroxyl group change the color of Uffelmann's reagent to yellow-greenish.

Method

Prepare Uffelmann's reagent in test tube by adding 1-3 drops of $FeCl_3$ solution to 2 ml 1% solution of phenol (**one reagent for all the students**). Mix, and add a few drops of the reagent to 1 ml of lactic acid solution. Lactic acid solution changes the violet color of the reagent to light yellow-greenish.

4.6.5 Salicylic acid detection

Method: Add to test tube 1 ml of water, a few crystals of salicylic acid and 1 drop of $FeCl_3$ solution. Observe the violet color, similar to previously performed $FeCl_3$ reaction with phenol solution.

4.7 Experiment No. 7 Detection of unsaturated fatty acids

4.7.1 addition reaction with iodine solution

Unsaturated fatty acids react with iodine (and other halogens) discoloring the iodine solution and yielding iodine addition products.

1. R-CH=CH-COOH + I_2 → R-CHI-CHI-COOH

Method: Add a few drops of Hüble's reagent (iodine in alcoholic solution of $HgCl_2$) to 1 ml of olive oil (oleic acid), and shake. After a few minutes the solution becomes colorless.

4.7.2 Oxidation reaction

Double bonding in unsaturated fatty acids is sensitive to oxidation. In the presence of oxidizing reagents, the molecule of unsaturated fatty acid, in the place where double bonding exists, is broken into two molecules of aldehyde. This process can be observed after a few days' exposition of fat to open air and sunlight (rancid process).

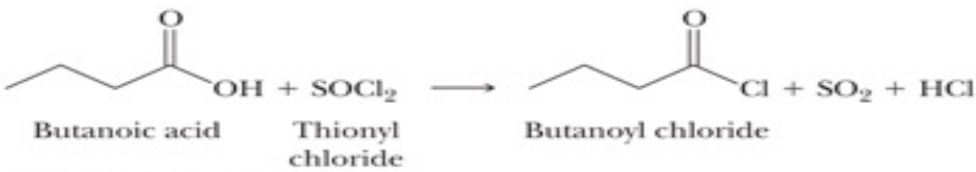
Method

Add 3 drops of olive oil to 1 ml of sodium carbonate (Na_2CO_3) solution and then a few drops of potassium permanganate solution ($KMnO_4$), and mix. After a few minutes, the permanganate solution becomes colorless.

4.7.3 Acid Chlorides:

The functional group of an acid halide is a carbonyl group bonded to a halogen atom. Among the acid halides, acid chlorides are by far the most common and the most widely used. In older nomenclature, these compounds were known as acyl halides. Acid chlorides are most often prepared by treating a carboxylic acid with thionyl chloride. The functional group of an acid halide is an acyl group bonded to a halogen. The most widely used are the acid chlorides. To name, change the suffix

-ic acid to ***-yl chloride***.



General Reactions of Acid Chlorides: Since acid chlorides have the best leaving group of acid derivatives, they react readily with a wide range of nucleophiles to form nucleophilic substitution products. HCl is usually formed as a by-product. A weak base like pyridine is added to the reaction mixture to remove the strong acid (HCl), forming an ammonium salt.

Method

Acid or Acyl Chloride RCOCl Fumes in air forming $HC_{l(g)}$	(1) Add a few drops to water, test with litmus and silver nitrate solution. (2) Add to a little ethanol and pour the mixture into water.	(1) Litmus turns red and a white precipitate with silver nitrate. (2) As above and you may detect a 'pleasant' ester odor.

1. $RCOCl + H_2O \rightarrow RCOOH + HCl$

The acid chloride is hydrolysed to form HCl acid (chloride ions) and the original carboxylic acid.

2. $CH_3CH_2OH + RCOCl \rightarrow RCOOCH_2CH_3 + HCl$

An ethyl ester and hydrogen chloride are formed.

Table 4.8: General Reactions of Amides and Amines

	Boil the suspected amide with dilute sodium hydroxide solution, see in inorganic for ammonia tests.	ammonia evolved on boiling (no heat required to form ammonia, if it was an ammonium salt)
Acid Amide chemical test $RCONH_2$	Boil the suspected amide with dilute sodium hydroxide solution, see in inorganic for ammonia tests.	ammonia evolved on boiling (no heat required to form ammonia, if it was an ammonium salt)
Aliphatic amines (primary, where R = alkyl) chemical test $R–NH_2$ e.g., $CH_3CH_2CH_2–N_H2$	(1) Lower members soluble in water but a very fishy smell. Test with red litmus and conc. HCl(aq) fumes. (2) If a suspected salt of an amine, then add sodium hydroxide solution to free the amine.	(1) A fishy odor, litmus turns blue, white clouds with HCl. (2) The above is not observed until after adding the alkali.
Aromatic amines chemical test (where R = aryl with the amine or amino group directly attached to an aromatic ring) $R–NH_2$ e.g. $C_6H_5–N_H2$	(1) Dissolve the primary aromatic amine in dilute hydrochloric acid at 5^oC and mix with sodium nitrite solution. (2) Add a phenol dissolved in dilute sodium hydroxide.	(1) It should be a clear solution with few, if any, brown fumes. (2) A colored precipitate [red – brown – yellow]

Reactions: Unless it's a liquid or solid, only the more freshly odor distinguishes it from ammonia. The reaction is e.g.

1. $RCONH_2 + NaOH \rightarrow RCOONa + NH_3$
2. $R–NH_3^+ + OH^- \rightarrow R–NH_2 + H_2O$

(1) If a primary aromatic amine, a 'stable' diazonium salt is formed. Diazonium salts from aliphatic amines decompose rapidly evolving colorless nitrogen.

(2) An azo dyestuff molecule is formed in a coupling reaction for instance $C_6H_5–N=N–C_6H_4–OH$.

4.8 Experiment No. 8 Halogenoalkanes reactions (haloalkanes)

R–X where R = alkyl, X = Cl, Br or I. The halide is covalently bound (C–X bond), so the halogen X cannot react with the silver ion to form the ionic $Ag^+X^-_{(s)}$ precipitate until it is converted to the 'free' X^- ionic form. Note that aromatic halogen compounds where the X is directly attached to the ring do NOT readily hydrolyze in this way and no AgX ppt.

Table 4.9: General Reactions of alkyl halides

R–X where R = alkyl, X = Cl, Br or I	(1) Warm a few drops of the haloalkane with aqueous ethanolic silver nitrate solution, the ethanol increases the solubility of the immiscible haloalkanes. (2) Gently simmering a few drops with aqueous NaOH (may need to add ethanol to increase solubility and reaction rate). Add dilute nitric acid followed by aqueous silver nitrate solution.	(1) Observe color of precipitate and the effect of ammonia solution on it (for rest of details see the (1) notes for chloride, bromide and iodide tests above in inorganic) (2) See the (1) notes as above for more details.

(1) $AgNO_3 + RX\ R–NO_3 + AgX_{(s)}$

(2) The sodium hydroxide converts the halogen atom into the ionic halide ion in a hydrolysis reaction.

1. $RX_{(aq)} + NaOH_{(aq)} \rightarrow ROH_{(aq)} + NaX_{(aq)}$
2. then $Ag^+_{(aq)} + X^-_{(aq)} \rightarrow AgX_{(s)}$

The addition of dilute nitric acid prevents the precipitation of other silver salts or silver oxide (e.g. Ag_2O forms if solution alkaline).

4.8.1 Esters chemical test RCOOR'

Esters are derived from acids by replacing the –OH group with –OR group. Esters are named in a manner analogous to salts (e.g., methyl acetate, ethyl acetate, ethyl benzoate). Esters are prepared by esterification from carboxylic acids or better from their functional derivatives, because they are more reactive than acids. Many esters are rather pleasant-smelling substances with flavor and fragrance of many fruits and flowers and found in fats and oils responsible for the aroma and flavor of bananas, oranges, and strawberries. Mixtures of esters are used as perfumes and artificial flavors. The functional group of an ester is an acyl group bonded to -OR or -OAr. Name the alkyl or aryl group bondless to oxygen (instead of a hydrogen) followed by the name of the acid. Change the suffix ***-ic acid to -ate.***

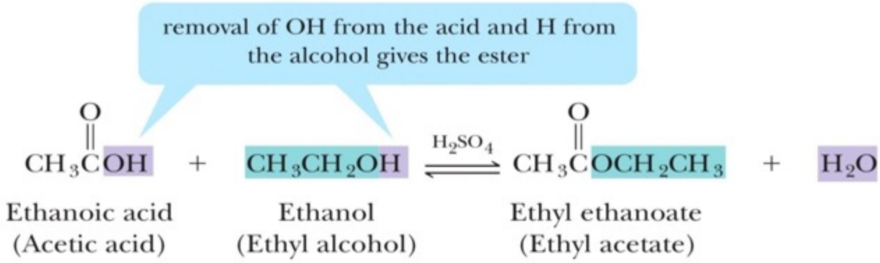


Table 4.10: Reactions of alkyl halides and iodoform

R = H, alkyl or aryl R' = alkyl or aryl There is no simple test for an ester. Usually, a colorless liquid with a pleasant 'odor'.	The ester can be reacted with saturated ethanolic hydroxylamine hydrochloride + 20% methanolic KOH and gently heated until boiling. Then mixture acidified with 1M $HCl_{(aq)}$ and $FeCl_{3(aq)}$ added drop wise.	Deep red or purple color formed. The test depends on the formation of a hydroxamic acid R–C(=NOH)OH which forms colored salts with $Fe^{3+}_{(aq)}$ ion.
Iodoform test The formation of CHI_3, triiodomethane (or old name 'iodoform'.	$NaOH_{(aq)}$ is added to a solution of iodine in potassium iodide solution until most of the color has gone. The organic compound is warmed with this solution.	A yellow solid is formed with the smell of an antiseptic, CHI_3, tri–iodomethane, melting point 119^{o}C.

Reactions:

1. The reaction is also given by acid chlorides and acid anhydrides, and phenols give a purple color with iron (III) chloride.
2. This reaction is given by the alcohol ethanol CH_3CH_2OH and all alcohols with the 2–ol structure –CHOH–CH_3 and the aldehyde ethanal CH_3CHO and all ketones with the 2–one structure R–CO–CH_3 (methyl ketones).
3. It's a combination of halogenation and oxidation and is not a definitive test for anything; it just indicates a possible part of a molecules structure.

Chapter

5

Biochemistry Experiments

Chapter Five

Biochemistry Experiments

5. Introduction and scope of biochemistry

Biochemistry is the branch of life science which deals with the study of chemical reactions occurring living cells and organism. Descriptive biochemistry deals with the qualitative and quantitative characterization of the various cell components. The major objective of biochemistry is the complete understanding of all the processes associated with living cells as the molecular level. To achieve this biochemist have attempted to isolate molecules, determine, analyze the function, illustrated aspect of disease … etc.

Theory: Carbohydrates are optically active polyhydroxy aldehydes, and ketones or compounds, which give these units as hydrolysis product. Starch, cellulose and sugars are the familiar examples of carbohydrates. Carbohydrates are classified on the basis of number of polyhydroxy aldehyde or ketone units obtained from them on hydrolysis. Three broad classes are as follows:

(i) Monosaccharides: These cannot be hydrolysed further to polyhydroxy aldehydes or ketones.

(ii) Oligosaccharides: These yield 2-10 monosaccharide units on hydrolysis. Common amongst these are disaccharides, which produce two monosaccharide units.

(iii) Polysaccharides: These yield large number of monosaccharide units on hydrolysis. Monosaccharides are further classified on the basis of number of carbon atoms and functional group present in them. If a monosaccharide contains aldehydic group it is called aldose. If it contains keto group, it is called ketose. Carbohydrates of all classes give Molisch's test. Carbohydrates, which are sweet in taste, are called sugars. Glucose, fructose (fruit sugar) and sucrose (table sugar) are examples of sugars. Sugars are classified into two major categories: reducing sugars and non-reducing sugars. Reducing property of sugars is detected by the three tests namely Fehling's test, Benedict's test and Tollen's test. Carbohydrates are the main source of energy in most organisms. They take part in cell membrane and cell wall formation. They also play an important role in lubrication, cellular inter communication, and immunity. Some common disaccharides are:

- ✓ Glucose + Glucose = Maltose
- ✓ Glucose + Glactose = Lactose
- ✓ Glucose + Fructose = Sucrose

1. Homo-polysaccharides and Hetero-polysaccharides

They are made up of the same kind of monosaccharide units. E.g. starch, glycogen, and cellulose. They are polymers of glucose and inulin (polymer of fructose). They are made up of different kinds of monosaccharide units, and are further divide into two main groups:

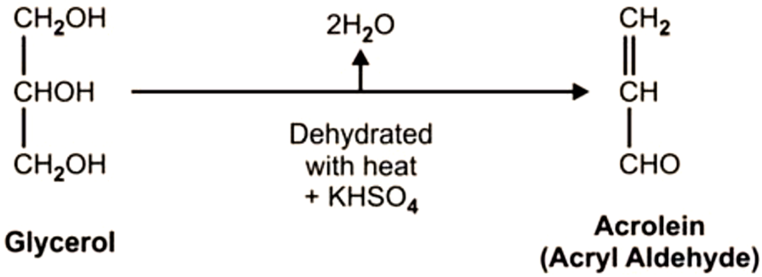
- ➢ **nimal origin:** e.g., mucoplysaccharide group which includes hyaluronic acid, heparin, chondriotin sulphate, blood group, and serum mucoids.
- ➢ **Plant origin***:* The most common example is the mucilage group which includes agar, vegetable gums and Dectins. In plant cell walls, cellulose occurs in densely packed fibrils called hemicelluloses.

1. Starch is stored in the form of glucose in plants.
2. Glycogen is stored in the form of glucose in Animals.
3. Cellulose is a structural polysaccharide of plant cells. Although it is present in the human diet, but it cannot be digested because humans lack the enzyme cellulase.

2. Glycerol

Test *Acrolein test:* The presence of glycerol is detected by acrolein test. Glycerol when dehydrated with heat and $KHSO_4$ produces ***"acryl aldehyde"*** which has pungent or acrid odor.

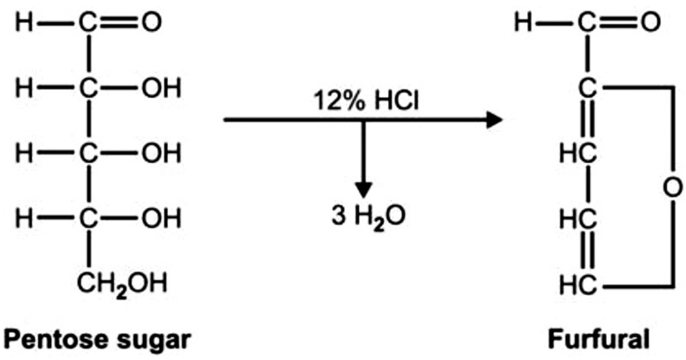
Industrial: Glycerol finds many uses in industry, as a result of its solubility, its solvent action and its hygroscopic nature. Many pharmaceuticals and cosmetic preparations have glycerol in their formulas.



Application:

1. The reaction is used for the quantitative determination of pentoses and compound carbohydrates containing pentoses. Furfural can combine with **phloroglucinol** to form a relatively insoluble compound, **furfural phloroglucide**, which may be used in estimating the furfural formed in the reaction as a measure of the pentose present.

2. Hexoses are decomposed by hot strong mineral acids to give hydroxymethyl furfural, which decomposes further to produce laevulinic acid, formic acid, CO and CO_2. The furfural products thus formed by decomposition with strong mineral acid can condense with certain organic phenols to form compounds having characteristic colors. Thus, it forms basis for certain tests used for detection of sugars see the equation below.

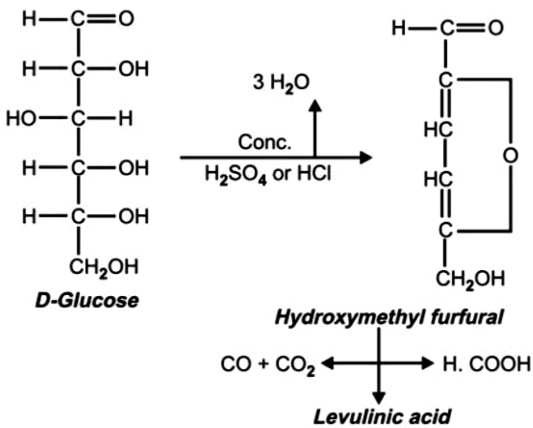


3. Action of acids on carbohydrates

Polysaccharides and the compound carbohydrates in general are hydrolyzed into their constituent monosaccharides by boiling with dilute mineral acids (0.5 to 1.0 N) such as HCl or H_2SO_4.

• With concentrated mineral acids the monosaccharides are decomposed.

• ***Pentoses*** yield the cyclic aldehyde ***"furfural"***. Twelve percent (12%) HCl has been found most satisfactory for decomposition as following equation.



4. Reducing Sugars and Non-reducing sugars

Reducing sugars are those which have potentially an aldehyde and ketone group in their structure. In other words, these sugars have a free anumeric carbon atom; by virtue of this, they have the ability to give a H atom to other substances.i.e., hey have the ability to reduce other substances and themselves get oxidized. All monosaccharides i.e., glucose, fructose, and glactose are reducing sugars because they have a free carbon atom. Disaccharides as maltose and lactose are also reducing sugars. Their anumeric carbon atoms are engaged in making bonds with each other; therefore, they don't have a free aldehyde or ketone group in their structure, and thereby cannot reduce other substances. Some disaccharides like sucrose and all polysaccharides are non-reducing sugars. However, on hydrolysis with an acid, base, and an enzyme, they yield smaller units which have a reducing capacity.

Reducing action of sugars in alkaline solution

All the sugars that contain free sugar group undergo enolisation and various other changes when placed in alkaline solution. ***The* enediol *forms of the sugars are highly reactive*** and are easily oxidised by O_2 and other oxidising agents and forms sugar acids. As a consequence, they readily reduce oxidising ions such as Ag^+. Hg^+, Bi^{+3}, Cu^{+2} (cupric) and $Fe(CN)_6^{-3}$.

Application: This reducing action of sugars in alkaline solution is utilized for both qualitative and quantitative determinations of sugars. Reagents containing Cu^{+2} (ic) ions are most commonly used. These are generally alkaline solution of cupric sulphate containing

• Sodium potassium tartarate **(Rochelle salt)** and strong alkali NaOH/KOH as in **Fehling's solution (not used now).**

• Sodium citrate and weak alkali sodium carbonate as in

Benedict's Qualitative reagent.

which dissociate sufficiently to provide supply of readily available Cu^{+2} (cupric) ions for oxidation.

• The alkali of the reagents enolises the sugars and thereby causes them to be strong reducing agents. ***Enolization is better in weak alkali than strong alkali.***

Reaction: When a solution of reducing sugar is heated with one of the alkaline copper reagents, the following reactions occur (given in box below).

The Cu^{+2} (ic) ions take electrons from the enediols and oxidize them to sugar acids, and are, in turn reduced to Cu^{+} (ous) ions. The Cu^{+} (ous) ions combine with $–OH^{–}$ ions to form yellow cuprous hydroxide, which upon heating is converted to red cuprous oxide. The appearance of a yellow to red precipitate indicates reduction and the quantity of sugar present can be roughly estimated from color and amount of precipitate.

5.1 Experiment No. 1 Hydrolysis of Polysaccharides

Theory of test for Polysaccharides (Starch): Starch gives blue color with iodine solution due to the formation of a complex known as starch iodide complex. Starch is present in wheat, rice, maize, potatoes. etc. Heating of starch in the presence of conc. HCl causes its hydrolysis into glucose. Because glucose have free Aldehyde group, therefore it is a strongly reducing monosaccharide, and hence Benedict's, Selivanoff's and Osazone tests become positive.

- Sodium carbonate is added to neutralize excessive HCl, because the reducing ability of reducing sugars is high in alkaline medium, and hence gives good results of Benedicts, Selivanoff's and Osazone tests.
- Erythrodextrin give red color. Its further hydrolysis produces achrodextrins, which gives negative iodine, test. When the iodine test becomes negative, we heat test tubes for two minutes more. The reason being is to provide time to complete hydrolysis of achrodextrin into maltose and maltose into glucose.

Objective: It demonstrates the hydrolysis of starch into glucose.

1. Polymers are broken down by hydrolysis which is essentially the reverse of condensation.
2. An –OH group from water attaches to one monometer and H attaches to the other.
3. This is a hydrolysis reaction because water (hydro) is used to break (lyse) a bond.
4. When a bond is broken, energy is released.
5. Polysaccharides such as starch, dextrin and glycogen, give positive iodine test.

6. Starch is a non-reducing polysaccharide therefore it does not give positive result with Benedict's, Fehling's and Barffoed's reagents, nor does it form any Osazone.
7. However, after hydrolysis into monosaccharide by the actions of strong acid, its components (glucose molecules) give the entire test positive.

Glycogen and starch are made up of amylose (1,4 glycosidic linkages) and amylopectin (1,6 glycosidic linkages). Starch has more of 1,4 glycosidic linkages compared to glycogen which has more branched chains with 1,6 glycosidic linkages. Glycogen is a storage polysaccharide in mammalian liver and muscles. Starch, on hydrolysis by acid (conc. HCl) gives the following products.

Starch → soluble starch → amylodextrins→ erythrodextrins → achrodextrins → maltose →glucose. On enzyme (amylase) hydrolysis, maltose is the major end product.

Table 5. 1 shows the Physical properties

Experiments	Observations	Results
1. Appearance	Opalescent	
2. Color	White	
3. Odor	Odorless	
4. iv. Reaction to litmus	No change	Neutral

Table 5. 2 shows the Chemical Tests

Experiments	Observations	Results
1. a. To 3 ml of sample solution, add 2 drops of 0.05 N iodine solution b. Heat the solution c. Cool the solution	a. Deep blue color b. blue color disappears c. Color reappears	Starch present

2. To 3 ml of sample solution, add 1 ml 40% NaOH, mix and add few drops of 0.1 N iodine solution	No color	Iodine is converted to sodium iodide and sodium Iodate. Hence, free iodine is not available for starch
To the above solution add 1 ml conc. HCl	Color reappears	Acid neutralizes the alkali to release free iodine, which can combine with starch
3. Benedict's test	No colored PPT	Starch is a non-reducing carbohydrate.
4. Rapid hydrolysis of starch and Benedict's test a. To 5 ml of sample solution add 6 drops of conc. HCl, boil for 1 min, cool, add 6 drops of 40% NaOH b. To 5 ml of Benedict's reagent, add 1 ml of above neutralized solution, boil	Red PPT	Starch is hydrolysed by acid to give glucose Units.

Iodine-starch absorption complex is blue in color. The complex breaks on heating, and forms again on cooling. Sometimes color may not reappear on cooling as small amounts of iodine added may vaporize away during heating.

Experiments	Observations	Results
Iodine test Benedict test	No Color No colored ppt	Glycogen, starch, dextrin absent Glucose, fructose, lactose, maltose absent

Reagents for Iodine test:

1. Concentrated Hydrochloric Acid
2. Solid sodium carbonate
3. **2% iodine solution:** It consists of 2.5 gm iodine and 7.5 gm of potassium iodide, dissolved in 500 ml in distilled water. Iodine is added slowly until a deep yellow color is produced.
4. 10% NaOH.
5. Concentrated H_2SO_4

Methods of Iodine test

Make a suspension of (0.5 g) starch in 5 ml water and pour it in 50 ml boiling water to get an aqueous colloidal solution. To this add a few drops of aqueous iodine solution. The appearance of blue color indicates the presence of starch.

Test for Oils and Fats Theory

These are the esters of glycerol and long chain fatty acids and are known as triglycerides. Triglycerides which are liquids at room temperature are oils and those that are solids are called fats. Oils are of plant origin and fats are of animal origin. Triglycerides in which three acyl groups are same are called simple triglycerides and a triglyceride in which three acyl groups are different is called mixed triglyceride. Many naturally occurring fatty acids contain two or three double bonds. The fats from which these come are called polyunsaturated fats or oils. While oils are glycerides of unsaturated fatty acids. Fats and oils are insoluble in water. On heating with potassium hydrogen sulphate, oils and fats give characteristic odor of acrolein. This is the test for glycerol present either free or combined as an ester. On heating with potassium hydrogen sulphate, glycerol is dehydrated and acrolein is formed which has a pungent odor.

Method

Add a few crystals (0.5g) of dry potassium hydrogen sulphate to 3 ml of mustard oil/ghee taken in a test tube and heat the content of the test tube gently. A pungent smell confirms the presence of an oil or a fat.

Interpretation:

1. In test tube ‘C’ Benedict’s, Selvinoff’s test, Osazone test remain –ve because due to absence of conc. HCl in this test tube, the starch is not hydrolyzed. In this test tube iodine test will also remain +ve.
2. In test tube ‘T’ Benedict’s, Selvinoff’s test, Osazone test become +ve because due to presence of conc. HCl in this test tube the starch is hydrolyzed into glucose, which is a strongly reducing monosaccharide.
3. Although acid completely hydrolyze starch to give glucose but this process occurs through various stages. Before complete hydrolysis it gives various products, which react with iodine and produce different colors.

Table 5. 3 Stage of hydrolysis and their colors

Stage of hydrolysis	Color
(Starch (insoluble	Blue
(Starch (soluble	Blue
Amylodextrin	Blue purple
Erythrodextrin	Red
Achrodextrin	No color
Maltose	No color
Glucose	No color

Precautions: The hydrolysis of starch is a stepwise process which enseus immediately after placing test tube ‘t’ in boiling water bath. After heating for first one minute, no time should be wasted to start doing iodine test, otherwise, the intermediates products cannot be observed.

5.2 Experiment No. 2 Hydrolysis of Sucrose

General: Sucrose commonly found as table sugar, is a Di-Saccharide. A Di-Saccharide by definition is a carbohydrate that when broken down will yield its constitutive monosaccharides, which in the case of sucrose will yield Glucose and Fructose united by ά 1, 2 Glycosidic linkages.

Principle: Sucrose has been hydrolyzed in the presence of concentrated HCl into its reducing monosaccharide constituents, glucose and Fructose. Hence the test for reducing sugars, are positive. Because of the presence of fructose, Selivanoff's test is positive for the keto sugar fructose. Osazone hence gives formation of crystals of the reducing sugars Glucose and Fructose. Solid sodium carbonate is added for the purpose of neutralizing excessive HCl, and to create an alkaline medium which provides the optimum pH for the reducing ability of reducing sugars.

Addition of solid sodium bicarbonate gives the following reaction.

1. $Na_2CO_3 + 2\ HCl \rightarrow 2\ NaCl + H_2O + CO_2$ (yielded as gas)

Carbon dioxide is expelled as bubbles during the effervescence.

Clinical Correlation:

1. Digestion of sucrose in the body.
2. Di-ssacharidase deficiency.
3. Role of Sucrose when given as intra- venous.
4. Sources of sucrose.
5. Invert Sugar.
6. Structure and component linkage.

Objective: Sucrose is a non-reducing sugar (due to the absence of free anomeric carbon atom), which in turn is made up of components Glucose and Fructose that are strong reducing sugars (because of the presence of free anomeric carbon atom at positions 1 and 2 respectively). Sucrose hence does not yield a positive result when reacted with test for reducing sugars. However, if sucrose if broken down (hydrolyses) to yield its constituent sugars, it will yield positive results whence reacted with test for reducing sugars:

1. Benedict's test and Fehling's test for reducing monosaccharides and disaccharides.
2. Selivanoff's test for keto-hexoses, namely fructose.
3. Osazone crystals, namely Glucosazone crystals.

Physical Characters: Sample: 1% glucose or 1% fructose solution given separately.

Experiments	Observations	Results
1. Appearance. 2. Color 3. Odor 4. Reaction to litmus	Clear Colorless Odorless No change	Neutral

Reagent required for Hydrolysis of sucrose

1. Concentrated Hydrochloric acid.
2. Solid Sodium carbonate.

Test to confirm hydrolysis

1. Benedict's Test.
2. Selivanoff's Test.
3. Osazone Test.

Method

Take 2 test tubes and label them as **'T'** and **'C'** for test and control sample respectively. Take 15 ml of sucrose in both test tube T and C. Add to test **T** 10 drops of concentrated HCL, and 1 ml of distilled water to test tube **C**. Heat both the test tube for 2 minutes and then allow them to cool in room temperature. Add a very small amount of solid sodium carbonate in both the test tubes, and continue to do so till the effervescence stops. Now from both the test tubes make 3 samples and performs in each of the test tubes

Benedict's test. Selivanoff's test, and Osazone test, to confirm hydrolysis.

Interpretation:

1. Positive result for Benedict's shows that it is a reducing sugar, which is yielded by Glucose and Fructose now present in the test sample.
2. Selivanoff's will yield a positive result, indicating the presence of a keto hexose namely Fructose, which is now present after hydrolysis.
3. Osazone test will yield crystals resembling bundle of grass, indicating the formation of Glucosazone and fructosazone crystals.

Control sample: The control sample in test tube **C** will not yield any results indicating the absence of Hydrolysis and reducing sugars.

5.3 Experiment No. 3 Iodine Test

Goal: To detect polysaccharide by Iodine test. Basically, it is a qualitative test for detection of polysaccharides. By this test we can differentiate between starch, dextrin and glycogen.

Principles:

1. This test depends upon the property of adsorption possessed by the large polysaccharide molecules. Helically coiled polysaccharides adsorb smaller molecules of iodine on their surfaces to form a complex of ill-defined chemical nature. At least eight monosaccharide units should be joined in linear chain to give the colored complex. The starch iodine, dextrin iodine and glycogen iodine complexes are blue, reddish purple and reddish brown respectively.
2. Alkalis disrupt the secondary valencies of polysaccharides thus iodine is released from their surface therefore the color disappears. The strong acid restores the secondary valencies and helps in adsorption of iodine, thus solution again gains its respective color.
3. On heating the iodine and polysaccharide complex dissociates and therefore color disappears. On cooling, the complex is reformed and the color reappears except in the case of dextrin. It has been suggested that

heating produces a change on the surface of the dextrin molecules as a result of which they loss the property of adsorption, therefore, color does not reappear on cooling

Reagents:

1. 2% Lugo's iodine solution: It consists of 2.5 gm iodine and 7.5gm of potassium iodide dissolved in 500 ml distilled water. Iodine is added slowly until a deep yellow color is produced.
2. 10% NaOH
3. Concentrated HCl
4. Concentrated H_2SO_4

Method

Take 3ml of carbohydrate solution in a test tube. Acidify it with one drop of concentrated HCl. Now add one small drop of iodine solution in this test tube and mix it. Because excess of iodine may give false result, therefore, it is better to use a glass rod to obtain small quantity of iodine. Dip the tip of glass rod in the iodine solution. The tip will carry very small amount of iodine. Now mix this amount of iodine in the above test tube by dipping the tip of glass rod.

Interpretation:

1. Starch gives a blue color on addition of iodine.
2. Dextrin gives a reddish-purple color on addition of iodine.
3. Glycogen gives a reddish-brown color with iodine.
4. No change in color indicates the absence of polysaccharide in the given solution.

Method of effect of pH

1. Perform iodine test and add 10% sodium hydroxide drop by drop till the color disappears.
2. Now add concentrated sulphuric acid drop by drop till color reappears.
3. Iodine test is positive only in acidic medium.

Procedure of effect of temperature: Perform iodine test and heat the test tube till the color disappears. Now cool the test tube till color reappears (except in case of dextrin).

Interpretations: Iodine test is negative at higher temperature and positive at lower temperature. And it is better to perform it at or near the room temperature i.e. 20.

5.4 Experiment No. 4 Benedict's Test

Objectives: It is a qualitative and semi qualitative test for reducing carbohydrates. Benedict's test is more specific than Fehling's test. More over in Benedict's test uric acid and creatinine does not interfere its results.

Principle: Principle is similar to Fehling's test; the reducing sugar can reduce cupric ions to the cuprous ion, which is the basis of Fehling's and Benedict's test. The cupric hydroxide is then reduced to cuprous oxide on heating with reducing carbohydrates.

1. $CuSO_4 \rightarrow Cu^{++} + SO_4^{-2}$
2. $Cu^{++} + 2OH^- \rightarrow Cu(OH)_2$
3. $Cu(OH)_2$ + Na citrate → $Cu(OH)_2$: Na citrate complex
4. $Cu(OH)_2$: Na citrate complex → $2OH^- + Cu^+$ + Sodium citrate + oxidized sugar + reducing sugar
5. $Cu^+ + OH^- \rightarrow CuOH \xrightarrow{heat} Cu_2O$ (Red ppt)

Cuprous hydroxide during the process of heating is converted to red cuprous oxide, which precipitates immediately. The precipitation of cuprous hydroxide is avoided by sodium citrate. In Benedict's test alkaline medium is provided by sodium carbonate. Note that ultimate quantity of cuprous oxide produced at the end of the reaction depends upon the amount of reducing sugars present in the sample used. As benedict reagent has blue color the final color is the mixture of blue color of Benedict's reagent and red color of Cu_2O. It may vary from green to brick red depending upon the concentration of reducing sugar. If the sample contains the reducing sugar more than 2% the final color of solution will remain red, because production of red cuprous oxide will not affect the already present color of solution.

Reagents: Benedict's reagent consists of: Copper sulphate, Sodium citrate **and** anhydrous sodium carbonate**.**

Benedict's reagent is prepared by dissolving 173 g of sodium citrate and 90 g of anhydrous sodium carbonate in about 750 ml of distilled water. Slightly heat to dissolve the content and filter the solution. Dissolve separately 17.3 g of $CuSO_4$ in about 100ml of water and is then added to the solution of sodium citrate and sodium carbonate with continuous stirring. Finally, the volume of solution is made up to 1000ml with water.

Method of Benedict's test

Experiments	Observations	Results
To 5ml of Benedict's reagent add 8 drops of sample solution, boil for 2 min	Green-yellow to brown or orange-red ppt	Glucose and fructose reduces Cu^{2+} to Cu^{+} in alkaline medium on heating. In turn glucose is oxidized

1. Reducing sugars under alkaline conditions tautomerize and form enediols. The enediols are unstable and decompose to yield a variety of products. 1, 2-enediols will give formaldehyde and a pentose. The chain reaction continues to produce short chain aldehydes, which are powerful reducing agents. They can reduce cupric ion to cuprous form, which is the basis for the Benedict's (and Fehling's) reaction. In order to keep the hydroxide in solution, a metal chelator like citrate (or tartrate) is included in the solution

2. Benedict's reagent contains $CuSO_4$ (to provide cupric ions), Na_2CO_3 (to make the pH alkaline), and Nacitrate (chelates Cu^{2+} and releases it slowly for reduction), thus preventing the formation of black CuO. Sodium citrate acts as a stabilizing agent. Copper is reduced to produce green, yellow, orange or red precipitate.

3. It is frequently used for detecting sugar in the urine of diabetic patients. Many reducing substances in urine like ascorbic acid can also give positive test.

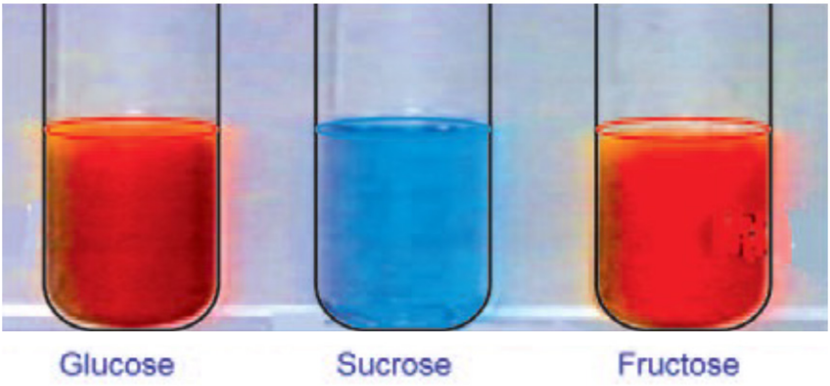


Figure 5.1: Benedict's test results colors

Interpretation: Appearance of green, yellow, orange or red precipitate indicates that carbohydrate is reducing one. This is semi qualitative test. If the solution is taken in correct proportion and procedure is followed strictly. The approximate concentration of carbohydrate can be judge from color of precipitate.

Fallacies: The test gives false positive result in the presence of some non-carbohydrate reducing agents i.e., ascorbic acids or end products of certain drugs like aspirin.

Clinical applications:

1. This is widely employed test for detection of glucose in urine.
2. It is commonly used for preliminary screening for hyperglycemia and for monitoring the effect of treatment.

Table 5. 4 Comparison between Fehling's and Benedict's tests

Fehling's test	Benedict's tests
1) The reagent is unstable	The reagent is very stable
2) It has to be prepared in two parts which have to be stored separately	Single solution is prepared and storage is convenient
3) It is only a qualitative test	It is both a qualitative and semi-quantitative test

4) The strong alkali (potassium hydroxide) present in the reagent can destroy base the destruction of carbohydrate	since sod carbonate is a very weak base the destruction of carbohydrate is insignificant
5) Auto reduction of cupric hydroxide occur resulting in false positive test	Auto reduction doesn't take place
6) It is more sensitive	6. It is less sensitive

Color of ppt.	Approximate conc. Of carbohydrate (gm %)
Green	0.1-0.5 (+)
Yellow	0.5-1.0 (++)
Orange	1.0-1.5 (+++)
Red	1.5-2.0 (++++)
Brick red	Above 2.0

5.5 Experiment No. 5 Fehling's Test

Fehling's Solution (deep blue colored) is used to determine the presence of reducing sugars and aldehydes. Perform this test with fructose, glucose, maltose and sucrose.

Objectives: It is a qualitative test for detecting reducing sugars.

Principle: Reducing carbohydrate can be detected by several tests based on their oxidation by certain metal ions e.g; copper, bismuth, silver, mercury. In Fehling's test the reducing sugar can reduce cuprous ions to cupric ions. Copper sulphate is present in Fehling's reagent hydrolyzes to give cupric hydroxide.

1. $CuSO_4 \rightarrow Cu^{++} + SO_4^{-2}$
2. $Cu^{+2} + 2OH^{-2} \rightarrow Cu(OH)_2$
3. $Cu(OH)_2$ + Na K tartrate → $Cu(OH)_2$: NaK tartrate complex
4. $Cu(OH)_2$:NaK tartrate complex → $2OH^- + Cu^+$ + Sodium citrate + oxidized sugar

5. $Cu^+ + OH^- \rightarrow CuOH \xrightarrow{heat} Cu_2O$ (Red ppt)

Cupric hydroxide on heating with reducing carbohydrate reduces to cuprous oxide (CuO). This color of solution changes from blue to red. The carbohydrate is simultaneously oxidized to corresponding aldonic acid (glucose to gluconic acid). Cupric hydroxide has a tendency to precipitate but sodium potassium tartrate prevents the precipitation of cupric hydroxide by forming a soluble deep blue complex with cupric ions. This complex dissociates to provide cupric ions for oxidation. The reduction occurs best in alkaline medium which is provided by potassium hydroxide.

Summary of Reaction:

1. Sugar + alkali → Enediol
2. Enediol + $Cu^{+2} \rightarrow Cu^+$
3. $Cu^+ + OH^- \rightarrow CuOH \xrightarrow{heat} Cu_2O$ (Red ppt).

Disadvantages and Advantages:

1. The reagent is unstable usually the storage time is 2 months.
2. It has to be prepared in two parts and stored separately.
3. The strong alkali present in reagent can destroy the carbohydrate.
4. Auto-reduction of CuOH may occur resulting in false positive test.
5. Because of these drawbacks Fehling is being replaced by another test known as Benedict's test. It is how ever sensitive test.

Reagents:

1. **Fehling's solution A:** It contains 7% copper sulphate solution which is prepared by dissolving 34.65g of $CuSO_4.5H_2O$ in 500 ml of distilled water.

2. **Fehling's solution B**: It contains potassium hydroxide and sodium potassium tartate (Rochelle salt). It is prepared by dissolving 125g of KOH and 173g of sodium potassium tartate in 500ml of distilled water.

3. **Working Solution:** Fehling solution A and B are mixed in equal volumes. It has a deep blue color.

Methods

Experiments	Observations	Results
Mix 1 ml of Fehling's. A solution to 1 ml of Fehling's B solution, boil, and add 1 ml of sample solution (boil again if necessary).	Green-yellow to or-nage-red to brown ppt	Glucose and fructose reduce Cu^{2+} to Cu^{1+} in alkaline medium on heating

1. The tartarate from Fehling's (B) solution chelates cupric ion, releasing it slowly for reduction thus preventing the formation of black cupric oxide.
2. $CuSO_4 + NaOH \rightarrow Cu(OH)_2 + Na_2SO_4$ reducing sugar Cu_2O (red ppt).
3. Since uric acid and creatinine also gives a positive test; Fehling's test is not commonly used nowadays.

Appearance of yellow or brick red precipitates indicates the presence of reducing carbohydrate.

5.6 Experiment No. 6 Molish's Test

Theory of Molisch's test

Molisch's Test is a sensitive chemical test for all carbohydrates, and some compounds containing carbohydrates in a combined form, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde (either furfural or a derivative), which then condenses with the phenolic structure resulting in a red or purple-colored compound. On adding concentrated sulphuric acid to the aqueous solution of carbohydrate containing alcoholic solution of 1-naphthol, a deep violet color appears at the junction of the two liquids. Concentrated sulphuric acid hydrolyses glycosidic bonds of carbohydrate to give monosaccharides which are dehydrated to an aldehyde known as furfural which undergoes reaction with 1–naphthol to give an unstable condensation product of deep violet color. This test may be given by some other organic compounds also.

Objective: To detect the presence of carbohydrate in the given solution by Molisch's test. It is a qualitative test for detection of carbohydrate in the given solution is positive for all carbohydrate whether free or bound to other substances such as protein (glycoprotein, myoprotein) or lipid (glycolipid).

Principle: Polysaccharide and dissacharides are hydrolyzed by concentrated sulphuric acid to mono-saccharides. These monosaccharides are dehydrated by conc. H_2SO_4 acid to form furfural or one of its derivatives. Furfural condense with α -napthol to form a violet color complex.

Molisch's Reagent:

1. This is prepared by dissolving 1 gm of α – napthol and 95 % ethyl alcohol making volume up to 100 ml.
2. Concentrated Sulphuric Acid:

Methods

Add 2-3 drops of alcoholic solution of 1% 1-naphthol in test tube '**A**' and then pour 2 ml conc. H_2SO_4 down the sides of the test tube so that it forms a separate layer at the bottom of the test tube. The formation of a purple ring at the interface of the two layers confirms the presence of carbohydrates. Appearance of reddish violet colored ring at the junctions of two liquid indicates the presence of carbohydrate in the test tube. In the presence of excessive α -napthol a green ring may also be seen; this should be ignored.

A. Fehling's test and Benedict test Black copper (II) oxide is formed on heating a suspension of copper hydroxide in alkaline solution.

$Cu(OH)_2 \rightarrow CuO + H_2O$

If some reducing agent is present in the reaction medium, then orange-red copper (I) oxide is precipated.

$2Cu(OH)_2 \rightarrow Cu_2O + 2H_2O + [O]$

Reducing sugars contain aldehydic group or a-hydroxy ketonic group, therefore in alkaline medium reduce Cu^{2+} ions. But if the reaction is carried out directly in the presence of an alkali then, copper (II) hydroxide gets precipitated. To overcome this problem, copper (II) ions are complexed with tartarate ions (Fehling's reagent) or citrate ions (Benedict's solution). Both the complex ions are soluble in alkaline medium and yield Cu^{2+} ions in such a low concentration that solubility product of cupric hydroxide is not reached. Reducing sugars react with Fehling's reagent according to the following reaction:

$$RCHO + 2\ Cu^{2+} + 5OH^{-} \rightarrow RCOO^{-} + Cu_2O + 3H_2O$$

The discharge of blue color due to Cu^{2+} ions and appearance of orange-red precipitate of Cu_2O, indicates the reducing property of sugars.

Method

Experiments	Observations	Results
To 2 ml of sample solution, add 1drop of a naphthol in alcohol and 2 ml conc. H_2SO_4 slowly and carefully along the side of the test tube	A purple ring develops	The sample contains carbohydrates

1. A strong dehydrating agent like conc. H_2SO_4 converts sugars to hydroxylmethyl furfural. The furfural condenses with phenolic compounds like a-naphthol to give the colored ring.
2. Molish test is given by at least five carbons.
3. α -naphthol in alcohol should be freshly prepared.
4. Water-acid interaction produces heat and can cause charring of carbohydrates, resulting in formation of a black ring. Therefore, acid should be layered very slowly and carefully to minimize this interaction. Excess α -naphthol solution also may give green ring.
5. Care must be taken while adding Molisch's reagent because in the presence of excessive α -napthol a green ring will be formed.
6. After formation of ring do not shake the test tube contents, it may distort the ring shape.

Tollen's test: Tollen's reagent is ammoniacal solution of silver nitrate. A reducing sugar, reduces silver ion to metallic silver which gets deposited on the inner surface of the test tube in the form of silver mirror. The reaction occurs as follows:

$RCHO + 2[Ag(NH_3)_2]^+ + 2OH^- \rightarrow 2Ag + RCOONH_4 + H_2O + 3NH_3$

Methods of Fehling's test

Mix 1 ml each of Fehling's solutions A and B in a test tube and add the mixture to test tube B. Heat the content of the test tube on a water bath. The formation of a orange-red precipitate indicates the presence of reducing sugar.

Methods of Benedict's test

Add 1 ml of Benedict's reagent to test tube C and heat the mixture to boiling in a water bath for 2 minutes. The formation of an orangered precipitate due to the formation of copper (I) oxide indicates the presence of reducing sugar.

Methods of Tollen's test

Prepare Tollen's reagent by adding sodium hydroxide solution dropwise to 1 ml aqueous silver nitrate solution to get the precipitate of silver oxide. Now add ammonium hydroxide solution while shaking the mixture so that initially formed silver oxide precipitate dissolves. Add the reagent to the sugar solution contained in a test tube and warm the reaction mixture on a water bath. Formation of silver mirror on the walls of the test tube shows the presence of reducing sugar.

5.7 Experiment No. 7 Baroede's Test

Barfoed's test

Barfoed's reagent, cupric acetate in acetic acid, is slightly acidic and is balanced so that is can only be reduced by monosaccharides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides. Perform this test with **glucose, maltose and sucrose.** Monosaccharides react with this reagent within 5 minutes to give a brick red precipitate of copper (I) oxide. Disaccharides take a longer time to react because aldehyde function is masked in the acetal linkage.

$RCHO + 2Cu^{2+} \quad RCOOH + Cu_2O$

The precipitate of cuprous oxide obtained is less dense and its color is brick-red instead of orange-red.

Objective: To determine whether the reducing sugars is a monosaccaride or disaccaride. This test is basically mean to detect monosaccaride in acidic medium. It can also be used to distinguish between monosaccaride and diasaccaride by controlling the time of heating.

Principle: This test differs from the Fehling's and Benedict's tests in aspect that the reduction of cupric ions is carried out in a mildly acidic medium. Aldoses and ketoses can reduce cupric ions even in acidic conditions. Since acidic medium is unfavorable for reduction, only the strongly reducing carbohydrates, i.e., monosaccharides, react very fast and give a positive test within three minutes. Disaccharides can also give this test positive provided they are boiled for sufficient time, enough to hydrolyze them in the presence of acidic medium.

Reagents: Barfoed's reagent consists of copper and glacial acetic acid acetate the reagent is prepared by dissolving 24 gm copper acetate in 400 ml of boiling water. To this add 25 ml of 8.5% glacial acetic acid solution. Stir and cool the solution and then add distilled water to make the volume 500 ml.

Methods:

Take 10 drops of 1% sugar solution in a test tube and add 1 ml Barfoed's reagent. Heat the content of the test tube in a water bath to boiling for five minutes. The formation of orange-red precipitate indicates positive test for monosaccharides. Disaccharides do not give this test.

Test for Sucrose

Hydrolyse the sucrose for performing the test by adding 5 drops of concentrated HCl to 5 ml of 1% sucrose solution and heating the mixture in a boiling water bath. Cool the mixture and add NaOH solution to obtain neutral or slightly alkaline solution. Perform the tests for reducing sugar and Seliwanoff's test given below with the hydrolysed product and record your results.

Or Take 2 ml of Barfoed's reagent and 2 ml of given solution in a test tube. Mix the contents thoroughly. Note the time by your watch. Place the test tube in a boiling water bath for five minutes. Remove the test tube from boiling water bath and cool under running tap water. Note the appearance of precipitates, if precipitates do not appear, put the test tube again in the boiling water bath for 15 minutes. And note the appearance of precipitates.

Barfoed's test	Observations	Results
To 5 ml of Barfoed's reagent add 2 ml of sample solution, Keep in boiling water bath for exactly 2 min	Fine red ppt. clinging to the walls of the test tube; some settles down on cooling	Mono-saccharides reduces reduce Cu^{2+} to Cu^{1+} in in acidic medium on boiling water bath for 2 min

The Barfoed's test depends on the concentration of sugar solution and the time of boiling also. A 5% disaccharides will also give Barfoed's test positive.

Rapid furfural test

Experiments	Observations	Results
To 2 ml of sample solution add 6 drops of a-naphthol in alcohol and 3 ml conc. HCl, boil for 30 sec exactly	Violet color within 30 sec of boiling, in Case of fructose.	Ketose (fructose) only responds

1. Conc. HCl converts hexoses to hydroxyl-methyl furfural. This conversion is faster for ketoses. The furfural condenses with a-naphthol to give the color. Prolonged boiling will give a positive test for aldose also.
2. The color develops within 30 sec of boiling. Sometimes, the color develops on keeping the tubes in the test tube rack for a few minutes.
3. This test can differentiate between glucose and fructose.

Interpretation: If the red precipitates appear at the bottom of test tube in five minutes. It indicates that the carbohydrates under test is a monosaccharide. If the reed precipitates appear after 15 minutes of heating, it indicates that the carbohydrates under test are a disaccharide.

5.8 Experiment No. 8 Selvinoff's Test

Seliwanoff's test: distinguishes between ketoses and aldoses of monosaccharides. Selvinoff's test is positive for ketose sugars hence is given positive for Fructose, Sucrose, and other fructose containing sugars. Ketoses dehydrate very rapidly under acidic conditions to give furfural, which reacts with resorcinol (1,3-dihydroxy benzene) to give a colored product. Monosaccharides are simple sugars, which: Cannot be hydrolyzed further. Have the formula $(CH_2O)_n$. Maybe either Aldoses or Ketoses.

1. **Aldoses:** It is a monosaccharide which contains Aldehyde groups. For example: Glyceraldehydes, Erythrose, Ribose, Glucose, and Glucoheptose.
2. **Ketoses:** It is a monosaccharide which contains ketose group. For example: Di-hydroxyacetone, Erythrulose, Ribulose, Fructose, and Sedheptulose.

Ketohexoses give red color and ketopentoses give blue-green color. Aldoses take longer time to produce color because under the same conditions, aldoses form furfural slowly, probably because b-elimination is required before dehydration to furfural.

Principle: Seliwanoff's Test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses. Perform this test with **glucose, fructose, maltose and sucrose.**

As per the property of carbohydrates, exposure of mono-saccharides to acids makes furfurals. The acid is provided by HCl in the Selvinoff's reagent. The Furfurals of ketohexoses made is 5-Hydroxymethyl Furfural. It condenses with Resorcinol to give a cherry red or pink colored complex. Sucrose will also give a positive test.

1. Fructose + HCl Hydroxymethyl Furfural + Levulinic Acid.
2. Hydroxymethyl Furfural + Resorcinol Cherry red complex.

Reaction of Acids: Monosaccharides are resistant to the action of dilute hot mineral acids. Strong acids remove the water and dehydrate hexoses to form Furfurals. When furfurals condense with phenols, they form a colored product.

Reagents: Selvinoff's reagents consist of: Selvinoff's reagent is prepared by dissolving 0.005g of Resorcinol in 50 ml of distilled water. Add 3 ml of concentrated HCl, in it very slowly. Add distilled water to make the total volume upto 100 ml.

Methods:

Add 2 ml of Seliwanoff's reagent to 10 drops of 1% sugar solution taken in a test tube. Heat the test tube in boiling water for two minutes. Ketohexoses give red color. Ketopentose gives blue-green color. Aldoses do not give color within two minutes. The final concentration of HCl in Selvinoff's should not exceed 12% HCl, because in the presence of very strong acid aldolases maybe converted to Ketohexoses and give a false positive test.

1. Similarly in the case with boiling, and hence should not be done for a prolonged period.
2. It goes without saying that other precautionary measures of the laboratory have to be taken.

5.9 Experiment No. 9 Phenyl Hydrazine Test (Osazone test)

Objective: This is a test for reducing carbohydrate, reducing dissacharides and many monosaccharides to be identified with the formation of osazone crystals. Hence this test is known as osazone formation test.

Principle: When the reducing carbohydrate are treated with phenyl hydrazine at 100 °c and P^H 4.3. A series of reactions take place resulting in formation of osazone of respective carbohydrate

1. Glucose + 3 phenyl hydrazine mol → Glucosazone + NH_3 + H_2O

A constant temp is provided by boiling water. A constant P^H is provided by buffer pair of sodium acetate and acetic acid. There action involves only 1st and 2nd carbon atoms of reducing carbohydrate. Since the glucose and fructose differ with respect to 1st and 2nd carbon atom. They form the same osazone. Mannose will form the same osazone. Galactose differ from glucose at carbon no 4. Therefore, its crystal shape is different. Sucrose and starch don't form crystals because they are non-reducing sugar. They can form crystals only when they are first hydrolyzed.

Reagents:

1. Phenyl hydrazine hydrochloride
2. Sodium acetate
3. Glacial acetic acid
4. Osazone mixture is prepared by mixing thoroughly one part on phenyl hydrazine hydrochloride and two parts of sodium acetate by weight.

Method

Take 5ml each of glucose, fructose, maltose and lactose solution in different test tubes and label them. Add about 0.3g of osazone mixture and 3 drops of glacial acetic acid in all test tubes. Put all test tubes n boiling water bath, observe each tube after every 5 mins till crystals appear. Note in each test tube how much time in min is been taken by the crystals to appear. If no crystals appear after 30 min remove these test tube from water bath and allow them to cool spontaneously, observe after every 5 mins till crystals appear up to 25 min. With the help of a glass rod take out some crystals on a glass slide, cover them with cover slip and observe under microscope.

Interpretation the results:

1. Yellow crystals in the test tubes containing glucose and fructose within 10 minutes of heating.
2. Galactose will give crystals within 20 minutes of heating.
3. Maltose will give crystals within 10-15 min of cooling.
4. Lactose will give crystals within 20-25 minutes of cooling.
5. Generally, monosaccharide give crystals on heating and all diasaccharide give crystals on cooling.

5.10 Experiment No. 10 Analysis of Disaccharides

All these three disaccharides can be hydrolysed into their respective monosaccharide units. Maltose is the product of enzyme hydrolysis of starch. Lactose is found in milk. 1% Lactose, 1% maltose, and 1% sucrose solutions are separately provided.

1. Maltose = Glucose + Glucose (-1,4 glycosidic linkage)
2. Maltose = Glucose + Glucose (-1,4 glycosidic linkage)
3. Lactose = Galactose + Glucose (-1,4 glycosidic linkage)
4. Sucrose = Glucose + Fructose (-1,2 glycosidic linkage)

Physical Characters

No.	Experiments	Observations	Results
1. 2. 3. 4.	Appearance Color Odor Reaction to litmus	Clear Colorless Odorless No change.	Neutral

Methods

Experiments	Observations	Results
Molish test (+ve for all carbohydrate). To 2 ml of sample solution, add 1drop of a-naphthol in alcohol and 2 ml conc. H_2SO_4 slowly and carefully along the side of the test tube	Violet ring at the junction of two liquids	All three disaccharides are dehydrated to give furfural, which condense with a-naphthol to give the colored ring
Fehling's test	a. Red ppt in case of lactose or maltose b. No characteristic ppt for sucrose	a. Lactose and maltose are reducing sugars b. Sucrose is a non-reducing sugar
Benedict's test	a. Red ppt in case of lactose or maltose b. No characteristic ppt for sucrose	a. Lactose and maltose are reducing sugars b. Sucrose is a non-reducing sugar.
Barefoed's test	No red ppt	Disaccharides do not respond to this test

1. Sucrose does not contain any free reducing group.
2. Higher concentration of sugar tends to give a Barfoed's positive test.
3. Prolonged boiling for more than 2 min in Barfoed's test may give positive test.

Detection of Unknown Carbohydrate

a. Physical Characteristics: (a) Appearance, (b) Color, (c) Odor, (d) Reaction to litmus

b. Chemical Tests: It is advisable to do 3 more tests as follows, to be sure of absence of carbohydrates in unknown solution. See the chart below

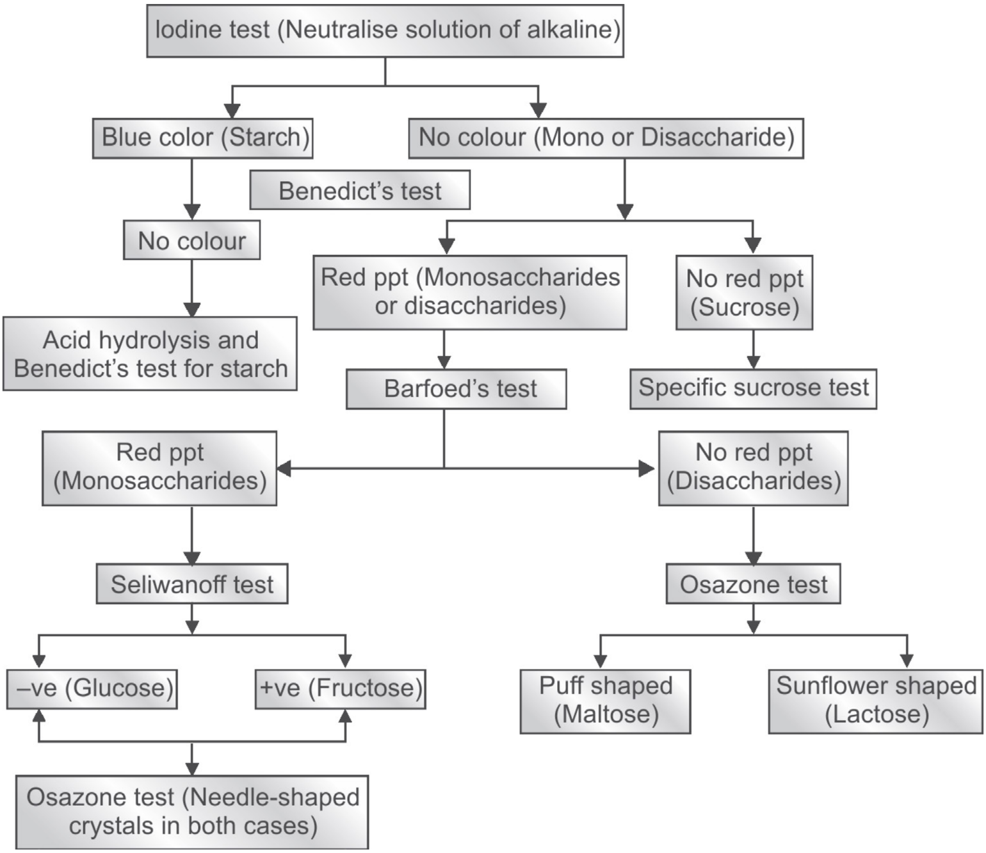


Figure 5.2 chart of distinguish between sugars

5.11 Experiment No. 11 Determine Acid Value of Oil Sample

Objective: To Determine Acid Value of Given Oil Sample.

Principle: The acid value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acids present in 1gm of substance. It is also known as neutralization number or acid number. Rancidity may occur in oil upon storage especially when oil contains high content of fatty acid or fatty oils. The decomposed compounds such as free fatty acids, peroxides, low molecular weight aldehyde and low molecular weight ketones are produced.

Table 5.5 Illustrate the Theoretical Acid Value of some Oil in (mg/100gm)

Oil Sample	Acid Value (mg/100gmof sample)
1. Grand (Groundnut)	0.28+/-0.00
2. Olive	3.42+/-0.01
3. Sunola (Cotton)	1.04+/-0.08
4. Grand (Soya)	0.17+/-0.00
5. Sunola (Groundnut)	2.70-0.10
6. Seasame	17.45-1-0.10
7. Coconut	1.99 to 12.8 (mg/1gm of sample)
8. Soyabean	01. to 0.2 (mg/gm of sample)
9. Refined sunflower	0.2 to 0.5 mg/gm of sample)

The acid value is calculated as

$$\textbf{The acid value of oil} = \frac{\text{Volume of KOH used (mL) x Normality of KOH}}{\text{weight of Oil Taken}}$$

The significance of the acid value

- The acidity of oil is due to hydrolysis or oxidation of oil by atmospheric moisture leading to the formation of fatty acids.
- Lubricants oil with acid values greater than 0.1 corrode metals, form gum and sludge during operation.

Apparatus: 250 ml Round Bottomed Flasks, Beakers, Reflux Condenser, Buchner flasks, Buchner funnel, Thermometer, Separatory Funnel, etc.

Chemicals: Oil sample, Potassium hydroxide, Ether, Ethanol, Phenolphthalein.

Method (a):

Unless otherwise specified in the individual monograph, dissolve about 10gm of the substance under examination, accurately weighed, in 50ml mixture of equal volume of ethanol (95%) and ether previously neutralized with 0.1 M KOH to phenolphthalein solution. if the sample does not dissolve in the cold solvent, connect the flask with reflux condenser and warm slowly with frequent shaking, until the samples dissolves. Add 1ml of phenolphthalein solution and titrates with 0.1M KOH until the solution remains faintly pink after shaking for 30 seconds. Calculate the acid value from the expression given above.

Method (b):

The accurately weighted quantity of oil is taken in a conical flask and 50 mi neutral alcohol is added, the mixture is heated over bath using water condenser for 1 hour. The contents are cooled titrated with KOH solution using phenolphthalein indicator.

Calculations:

$$\text{Acid Value} = \frac{5.61}{W}$$

where, n= the No. of ml of 0.1 M KOH required.

W= the weight in gram of substance.

Result: The Acid value

5.12 Experiment No. 12 Determine Saponification Value of oils.

Objective: To determine Saponification value of given oil sample.

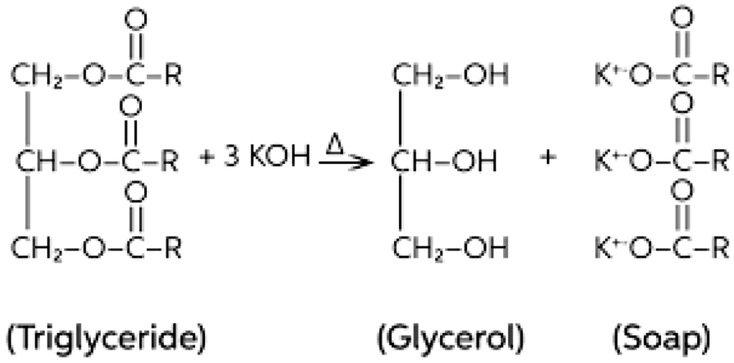
Principle: Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and he salt of the corresponding fatty acid. The saponification value is the number of milligrams of KOH necessary to neutralize the free acids and to saponify the ester present in 1 gm of the substance.

Table 5.6 Show the Theoretical Saponification Values of Some Oil

Fats / oils	Saponification value
Rapeseed oil	170-179
Menhaden oil	190.6
Corn oil	188-193
Olive oil	185-196
Soyabean oil	193
Cocco butter	193.55
Linseed oil	193-195
Butter	220-233
Coconut oil	246-260

Determination of saponification value accurately weighed quantity of oil is taken in a flask and 50 mL of 0.1 alcoholic KOH is added. The mixture is heated over water bath using water condenser for one hour. The contents are cooled and amount of un-reacted KOH in the flask is estimated by back titration with x N HCL using phenolphthalein indicator. Similarly, a back titration without oil sample is carried out.

Significance of saponification value: Petroleum oils do not saponify whereas vegetable and animals' oils have a characteristic saponification value. Saponification value indicates presences of vegetable and animal's oils additive in blended lubricating oils. Each blended oil has its own characteristics saponification value hence any deviation indicates changes of adulteration. Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NaOH) yield glycerol and potassium or sodium salts of fatty acids (soap).



Apparatus: 250ml Round bottom Flask, Beakers, Reflux Condenser, Buchner Funnel, Bruchner Flasks, Thermometer, Separatory Funnel etc.

Chemical: Oil sample, Potassium hydroxide, Ethanol (95%), Hydrochloric acid., Methyl red and Phenolphthalein.

Method

Unless otherwise specified in the individual monograph, introduce about 2gm of the substance under examination, accurately weighed into a 200ml flask of borosilicate glass fitted with a reflux condenser. Add 25ml of 0.5 M ethanolic KOH and a little pumice powder and boil under reflux on a water bath for 30 min. Add 1ml of phenolphthalein solution and titrate immediately with 0.5 M HCl. Perform a blank determination omitting the substance under examination. Calculate the saponification value from the expression given above.

Calculations: $$\text{Saponification Value} = \frac{28.05\ (b - c)}{W}$$

Where, w = weight in gram of the substance

Result: The Saponification value of ………..

5.13 Experiment No.13 Determine Iodine Value of Oil Sample

Objective: To Determine Iodine Value of Given Oil Sample.

Principle: The iodine value is the number which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100 gm of substance under the described conditions. It may be determined by any of the following methods. Unsaturated fatty acids can be converted into saturated by the process of hydrogenation. Depending upon the degree of unsaturation, the fatty acids can combine with oxygen or halogens to form saturated fatty acids. So, it is important to know the extent to which a fatty acid is unsaturated. There various methods for determining the unsaturation level of fatty acids, one of them is by determining the are iodine value of fats. iodine numbers are typically wont to determine the amount of unsaturation in fatty acids. This unsaturation is within the form of double bonds, which react with iodine compounds. The higher the iodine number, the additional C=C bonds are present in the fat.

This particular analysis is an example of ***iodometry***. A solution of iodine is yellow/brown in color. When this iodine solution is added to a solution to be tested, however, any chemical group (usually in this test C=C double bonds) that react with iodine effectively reduce the strength, or magnitude of the color (by taking iodine out of solution). Thus, the number of iodine needed to make a solution retain the characteristic yellow/brown color will effectively be used to confirm the amount of iodine sensitive groups present within the solution. The reaction related to this methodology of analysis involves formation of the di-iodo alkane (R and R' symbolize alkyl group or different organic groups). The precursor alkene (RCH=CHR') is colorless and so is the organo-iodine product (RCHI-CHIR').

In this method, the fatty acid is treated with an excess of the Wijs solution (iodine monochloride (ICl) in glacial acetic acid). Un-reacted iodine monobromide (or monochloride) is then reacting with potassium iodide, converting it to iodine, whose concentration can be determined by titration with sodium thiosulfate. It estimates the unsaturation of the fats and oils. Fatty acids react with iodine leading to the addition of the halogen at the C=C covalent bond site. During this reaction, iodine monochloride reacts with the unsaturated bonds to provide a dihalogenated single bond, of that one carbon has bound an atom of iodine.

After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.

1. $ICl + KI \rightarrow KCl + I_2$

This causes the remaining un-reacted iodine monochloride to form molecular iodine. The liberated $\mathbf{1}_2$ is then titrated with a standardized solution of 0.1N sodium thiosulfate.

2. $I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_2O_4$

Saturated fatty acids will not undergo halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is oil. For this reaction the liberated iodine will react with starch to give purple colored product and thus the endpoint can be observed so the starch is used as Indicator.

Important note:

1. Iodine monochloride is caustic. So handle the reagent with gloves.
2. For better results, perform the experiments without any time gap during addition of reagents as the liberated iodine is susceptible to oxidation by light.

Table 5.7 Show the theoretical Iodine values of some oil

Oil	Iodine value
Coconut oil	7.5-10.5
Olive oil	79-90
Palm oil	4-22
Sunflower oil	125-140
Ghee	26-38
Groundnut oil	84-100
Mustard Oil	98-110

Apparatus: 250 ml Round Bottomed Flasks, Beakers, Reflux Condenser, Buchner flasks, Buchner funnel, thermometer, separatory funnel, etc.

Chemicals: Oil sample, Potassium hydroxide, Ether, Ethanol, Phenolphthalein.

Iodine Monochloride Method or Wijs Method

Place an accurately weighed quantity of the substance under examination in dry iodine flask, add 10ml of Carbon tetra-chloride and dissolve. Add 20ml iodine monochloride solution, insert the stopper and allow standing in the dark at a temp. between 15-25°C for 30 min. Place 15ml of KI solution in the cup top carefully remove the stopper, rinse the stopper and sides of the flask with 100ml of water, shake and titrate with 0.1M sodium thiosulphate using starch solution, added towards the end of titration as indicator. Note the number of ml required.

1. Repeat the operation by excluding the substance under examination and note the number of ml required.

2. Calculate the iodine value from the expression.

Calculations:

$$\text{Iodine Value} = \frac{1.269\ (b - c)}{W}$$

where, w=weight of the substance.

Result: The Iodine value of ………………………..

5.14 Experiment No. 14 Qualitative Experiments of Color Reactions of Proteins

Principle: Proteins are made up of amino acid residues joined by peptide bonds. Due to their polypeptide structure and different amino acid residues, protein reacts with a variety of reagents to form colored products. These tests, known as color reactions of proteins, are of importance in qualitative detection and quantitative estimation of proteins, and of their constituent amino acids in body fluids and other biological materials. Proteins and amino acids used in different experiments:

1. Egg albumin is an egg protein, which is soluble in water.
2. Casein is the major protein in milk. It is a phosphoprotein with phosphate groups attached to the hydroxyl groups or serine and threonine residues. It is deficient in cysteine.

3. Gelatin is formed from collagen, the connective tissue protein, by boiling with water. It is a rich source of amino acid glycine. It is deficient in tyrosine, tryptophan and cysteine.
4. Metaproteins, proteoses and peptones are partially hydrolyzed products of proteins like albumins and globulins. Albumin has relatively low molecular weight. Gelatin, metaproteins, proteoses and peptones are derived proteins.

Color reaction of proteins based on peptide bonds and types of amino acid residues

Solution: 10% Egg-white or albumin.

1. **Biuret Reaction**

Experiments	Observations	Results
To 2 ml of sample solution, add 2 ml 5% NaOH and 3 drops of 1% CuSO4. Repeat the test with distilled water ((control	Purple-violet or pink color in test Blue color in control	Peptide linkages

1. The reaction is so named since biuret (NH_2-CO-NH-CO-NH_2) formed by the condensation of two molecules of urea when heated. CO-NH is the peptide linkage in biuret. At least two peptide bonds in the molecule are required for a positive test. $CuSO_4$ is converted to $Cu(OH)_2$ which chelates with peptide linkage in proteins to give the color.

2. Strictly avoid excess addition of $CuSO_4$. Mg and NH_4^+ ions will interfere in this reaction.

3. The color varies depending on the number of peptide linkages; albumin/ globulin give violet, proteoses purple and peptones dark pink color indicating that albumin/ globulins have largest number of peptide linkages.

2. Ninhydrin Reaction

Experiments	Observations	Results
To 1 ml of sample solution, add 0.1% ninhydrin solution, boil and cool	purple color	Amino acids present

1. All -amino acids give purple color. The imino acids, proline and hydroxyproline give yellow color. The colored complex is known as Ruhemann's purple. Glutamine and asparagine produce brown color.
2. ***alpha***-amino acid + ninhydrin → aldehyde + hydrindantin + NH3 + CO2; hydrindantin + NH3 + ninhydrin → Ruhemann's purple + $3H_2O$.
3. Proteins will give a faint blue color.
4. This reaction is often used to detect amino acids in chromatography.
5. Proteins do not give a true color reaction; but N-terminal amino group of a protein can react with ninhydrin to produce a faint blue color.

3. Xanthoproteic Reaction of Aromatic Amino Acids

Experiments	Observations	Results
To 2 ml of sample solution, add 1 ml conc HNO_3 and boil. Cool test tube and add 40% NaOH excess	Yellow precipitate Color of precipitate and the solution change to orange	Tyrosine, tryptophan or phenylalanine present

Yellow color is due to the formation of nitro derivatives of benzene ring containing amino acids (tyrosine and tryptophan), the color turns orange due to ionization when alkali is added. All proteins usually respond to this test. This reaction is also the basis of yellow stain in skin by nitric acid. Nitration of phenylalanine under these conditions normally does not take place.

4. **Modified Millon's Reaction (Cole's Test)**

Experiments	Observations	Results
To 2 ml of sample solution, add 2 ml 10% $HgSO_4$ in 10% H_2SO_4, boil, add 5 drops of 1% sodium nitrite, heat gently	Red PPT of mercury phenolate	Tyrosine present

1. The color is due to the formation of nitrated mercury phenolate ion of tyrosine (hydroxyphenyl group) present in proteins.
2. Heat coagulable proteins give red PPT, whereas smaller molecules of proteins like peptones give red colored solution without PPT.

5. **Test for Sulphur-containing Amino Acids**

Experiments	Observations	Results
To 2 ml of sample solution add 2 ml 40% NaOH, boil for 3 minutes, cool, and add 2–3 drops of lead acetate	Black or brown PPT	Cysteine or Cystine present

1. Avoid excess of lead acetate solutions, which will form white PPT.
2. Organic sulphur in cysteine and cystine are released as inorganic S2- ions which form lead sulphide as follows:

1. $R\text{-}SH + 2NaOH \rightarrow ROH + Na_2S + H_2O$
2. $Na_2S + (CH_3COO)_2Pb \rightarrow PbS + 2CH_3COONa$

Methionine does not give this test as the sulphur group in this amino acid is in thio-ether linkage, which is difficult to break. Albumin and keratin will answer this test, but casein (containing methionine) will not.

6. **Test for Phosphoprotein (Neumann's Test) (Casein Solution)**

Experiments	Observations	Results
To 5 ml of sample (casein) solution add 2 drops of .chlorophenol red indicator	Dark pink color	pH > 5.4
Add 1% acetic acid drop by drop. Decant the supernatant leaving only the precipitated casein in test tube.	Yellow color with maximum PPT	pH = 4.6, which is the isoelectric pH of casein
Add 12 drops of conc H_2SO_4, 4 drops of conc HNO_3. Heat the test tube continuously and slowly shaking it with caution.	Contents of test tube char and turn brown or black Color of digest changes to orange	
After no brown fumes are seen in the test tube, add 3 more drops of conc. HNO_3 and heat Repeat step II, 2 or 3 times until the liquid and fumes turn colorless after cooling, add 5 ml ammonium molybdate.	Color of digest changes from orange to yellow to colorless. Very fine canary yellow PPT	Organic P is converted to inorganic form (PO_4^{3-}) P is present in casein, it is a phosphor-protein

Notes: Air-cool the test tube keeping on test tube rack, not in cold water. Phosphorous bound with casein is released as inorganic phosphate by digesting with conc H_2SO_4 and conc HNO_3. This inorganic phosphate reacts with ammonium molybdate to produce canary yellow precipitate.

5.15 Experiment No. 15 Tests Based on Precipitation Reactions of Proteins

Tests for Proteins Theory

Proteins are complex organic compounds containing nitrogen and are made up of amino acids. Proteins are present in egg albumin, soya beans, pulses, fish, milk etc. Their presence can be confirmed by several tests. Due to the presence of characteristic side chains in them, certain amino acids exhibit typical color reactions that form the basis for their identification. Proteins also respond to the color reactions of amino acids, but can be distinguished from amino acids by biuret reaction, and coagulation reaction.

Proteins are large molecules with variable sizes, shapes and charges. They can be classified as simple, conjugated and derived proteins. Most simple proteins, especially globular proteins, when dissolved in water, form colloidal solution. Polar groups of the proteins (-NH_2, COO–, OH– groups) tend to attract water molecules towards them to produce a shell of hydration. Albumin has a greater degree of hydration than globulins.

1. Biuret test for peptide bonds

Alkaline copper sulphate reacts with compounds containing two or more peptide bonds to form complexes of violet color. The name of the test comes from the name of the compound, biuret, which gives this test. The reaction is not absolutely specific for peptide bond because many compounds containing two carbonyl groups linked through nitrogen or carbon atoms give a positive result.

Method of Biuret test

Prepare 0.5% (w/V) solution of casein or egg albumin in 0.1 M NaOH solution. Take 2-3 ml of the solution and add about 2 ml of 10% sodium hydroxide solution to it. Add a few drops of copper reagent and warm the mixture for about 5 minutes. Appearance of violet color due to the formation of a complex species of Cu^{2+} ions with - CONH – group confirms the presence of protein in the sample.

2. Ninhydrin reaction

Ninhydrin is a powerful oxidizing agent and reacts with proteins to give a blue-violet compound called Rhumann's purple.

Method of Ninhydrin reaction

Take 2-3 ml of an aqueous solution of egg albumin in a test tube. Add 3-4 drops of ninhydrin solution to it and heat. Appearance of blue color indicates the presence of protein.

3. Xanthoproteic reaction

Aromatic groups of either the free amino acid or protein, undergo nitration on heating with concentrated nitric acid. The salts of these derivatives are orange in color.

Method of Xanthoproteic test

Take 1 ml of an aqueous solution of egg albumin in a test tube and add a few drops of concentrated nitric acid. Heat the reaction mixture for a few minutes on a Bunsen flame. A yellow color appears. Cool the test tube under running tap and add a few drops of 10M sodium hydroxide solution, an orange color appears.

Precipitation by Salts

1. Supplied sample: 10% egg-white solution

Generally, proteins can be precipitated by the addition of salts. When an inorganic salt like ammonium sulphate is added to a solution of protein, it decreases concentration of water molecules available for stabilizing the protein solution and the protein is consequently precipitated. As globulin has higher molecular weight, lower concentration of salt is enough for its precipitation. Thus, globulins are precipitated at half saturation of ammonium sulphate or 22% sodium sulphate; but albumin will need full saturation of ammonium sulphate or 28% of sodium sulphate. Solubility of a protein depends on ionic concentration of the medium.

Table 5.7 Half Saturation Test with Ammonium Sulphate

Experiments	Observations	Results
1. To 3 ml of sample solution add equal volume of saturated ammonium sulphate solution, mix, let it stand for 5 minutes, then filter	White PPT	Globulins are precipitated by half saturation with ammonium sulphate
2. To 1 ml filtrate from Step I, add 1 ml 40% NaOH, and 1 drop 1% $CuSO_4$.	Violet color	Albumins are not precipitated by half saturation with ammonium sulphate
3. Take PPT in a test tube, add 2 ml 40% NaOH and 1 drop 1% $CuSO_4$	Violet color	Globulins are precipitated.

Filtrate contain high concentration of ammonium ions which interfere in biuret test by forming deep blue cupper ammonium ions $[Cu(NH_3)_4^{++}]$ which obscure the violet color produced by proteins. This can be overcome by the use of 40% NaOH and the test is called the modified biuret test.

2. Full Saturation Test with Ammonium Sulphate

Experiments	Observations	Results
4. To the filtrate from 1, add ammonium sulphate crystals in excess (some crystals should be left undissolved after thorough mixing), and filter	White PPT	Albumins are precipitated by full saturation with ammonium sulphate
5. To 1 ml filtrate from step 4, add 1 ml 40% NaOH, and 1 drop 1% $CuSO_4$	No violet color	Albumins are precipitated
6. Take PPT in a test tube, add 2 ml 40% NaOH and 11 drop 1% $CuSO_4$	Violet color	

1. Albumin and gelatin are precipitated by full saturation with ammonium sulphate; but peptones are not precipitated even by full saturation with ammonium sulphate, because they have smaller molecules.
2. Globulins are precipitated by 22% sodium sulphate and albumin by 28% sodium sulphate.

Supplied sample: 10% egg-white solution

Generally, proteins can be precipitated by the addition of salts. When an inorganic salt like ammonium sulphate is added to a solution of protein, it decreases concentration of water molecules available for stabilizing the protein solution and the protein is consequently precipitated. The process is known as “salting out”. Albumin tenaciously holds a large number of molecules of water and, therefore, needs a much higher concentration of salt than globulin to get precipitated. This property of proteins depends upon the type of amino acids that constitute them as well as their sizes and structures, and can be used for separating proteins from each other, such as albumin from globulins. As globulin has higher molecular weight, lower concentration of salt is enough for its precipitation. Thus, globulins are precipitated at half saturation of ammonium sulphate or 22% sodium sulphate; but albumin will need full saturation of ammonium sulphate or 28% of sodium sulphate.

3. Precipitation by Heavy Metals 10% Egg-White Solution

When the pH of a protein solution is higher than the isoelectric pH of the protein (generally in an alkaline medium), protein molecules become negatively charged anions. Positively charged heavy metal cations may then bind with the negatively charged protein anion, causing their precipitation. Salts of Fe, Cur, Zn, Pb, Cd and Hg are toxic, because they tend to precipitate normal proteins of the gastro intestinal wall. Raw egg is sometimes used as an antidote for mercury poisoning.

Methods:

Experiments	Observations	Results
1. To 2 ml of sample solution add 10% mercuric chloride solution drop by drop 2. To 2 ml of sample solution add 10% lead acetate solution drop by drop 3. To 2 ml of sample solution adds 10% ferric chloride solution drop by drop.	White PPT White PPT White PPT	Albumin/globulins are precipitated by heavy metals like Hg, Pb and Fe.

1. If the sample solution is significantly alkaline, its pH should be adjusted to 7–7.5 to avoid formation of metal hydroxides, which interfere with the test.
2. Avoid adding excess of heavy metal ions as this may re-dissolve the PPT due to absorption by the protein molecules, which will give them a positive charge.

Methods:

Table 5.8 Precipitation by strong Mineral acids (10% Egg White Solution)

Experiments	Observations	Results
I. To 2 ml of sample solution adds 2 ml conc HNO_3 slowly along the side of the test tube. II. To 2 ml of sample solution adds 2 ml conc HCl slowly along the side of the test tube.	White ring at the junction of two liquids White ring at the junction of two liquids	Albumin/globulins are precipitated by strong mineral acid

Test (I) is called Heller's test and is usually used to identify proteins in body fluids, particularly in urine.

4. Precipitation by Organic Solvents (10% Egg-White Solution)

Proteins in solution form hydrogen bonds with water. Organic solvents like acetone, ether or ethanol when added to a protein solution in water, reduce the concentration of water molecules available for keeping the proteins in solution and thus decrease the number of hydrogen bonds, precipitation and denaturation of proteins. This denaturation does not occur to some proteins at low temperature.

Experiments	Observations	Results
To 1 ml of sample solution add 2 ml ethanol and mix, repeat with acetone, ether or methanol.	Mild cloudy precipitate	Albumin/globulins are precipitated by organic solvents

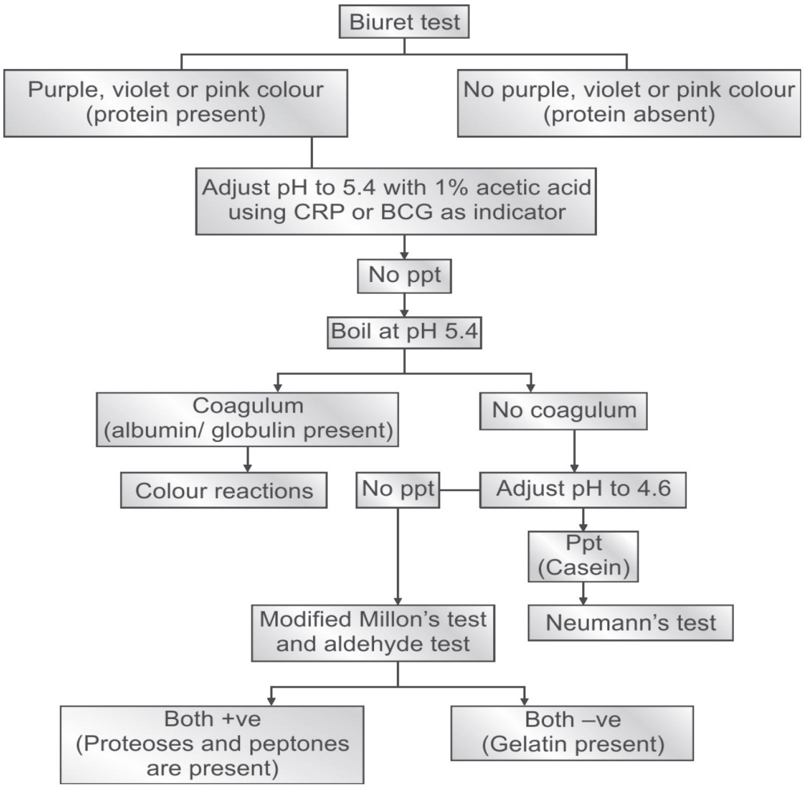


Figure 5.3 shows identification of an unknown protein in a solution

5.16 Experiment No. 16 Analysis of Urea and Uric acid

Urea is the end product of catabolism of proteins. It is excreted mainly in the urine. It is neutral in solution. One percent urea solution.

Table 5.9 Physical Character of urea

No.	Experiments	Observations	Results
1.	Appearance	Clear	Neutral
2.	Color	Colorless	
3.	Odor	Odorless	
4.	Reaction to litmus	No change	

1. Tests Method Hypobromite

Experiments	Observations	Results
To 3 ml of sample solution add few drops of alkaline hypobromite .solution	Effervescence of N_2	Urea present

1. $NH_2CONH_2 + 3NaOBr \rightarrow 3NaBr + N_2 + CO_2 + 2H_2O$
2. $2NaOH + CO_2 \rightarrow Na_2CO_3 + 2H_2O$

The test can also be done by using 1 ml 40% NaOH and a few drops of bromine water instead of NaOBr solution. Reagent must have sufficient bromine.

Methods

Experiments	Observations	Results
To 3 ml of sample solution add 3 drops of phenolphthalein indicator and a knife point of urease powder (or soyabean meal), mix and keep for 15 min	Solution turns pink due to alkalinity	Urea is hydrolysed to NH3 and CO_2, which form $(NH_4)_2CO_3$ to make the solution alkaline. Urease is the enzyme, which acts only on urea

Uric Acid

Uric acid is the end product of catabolism of nucleic acids in mammals. It is excreted mainly in the urine. A supplied uric acid is alkaline because it has to be dissolved in an alkali for preparing the solution. One percent uric acid solution.

Table 5.10 Physical properties of uric acid

No.	Experiments	Observations	Results
1.	Appearance	Clear	
2.	Color	Colorless	
3.	Odor	Odorless	
4.	Reaction to litmus	Reaction to litmus blue	Alkaline

Methods

Experiments	Observations	Results
1. To 3 ml of sample solution adds conc. HCl drop by drop.	Cloudy white precipitate	Uric acid is insoluble in acidic medium
Benedict's uric acid test to 5 ml of sample solution add 1 to 2 gm of anhydrous Na_2CO_3 powder and 5 to 6 drops of Benedict's uric acid reagent	Intense blue color	Uric acid reduces phosphomolybdic acid to blue phosphomolybdous acid

5.17 Experiment No. 17 Analysis of Milk

Milk is considered as a complete food with most of the nutrients needed for human body. It is the secretion of lactating mammary gland. The secretion during early stages of lactation is known as colostrum's.

The composition of milk is:

The pH of milk is 6.5 to 6.7. Its specific gravity averages 1.03, but changes depending on its dilution with water. The chief protein of milk is casein, which constitutes about 80% of the total milk proteins; the remaining are lactalbumin and lactglobulin. The proteins of human milk contain more arginine, tryptophan and sulphur containing amino acids. These proteins carry the immune bodies also, which are found in the blood serum. Thus, mother's milk transmits immunity to offspring. Milk fat exists as an emulsion due to presence of proteins in milk. About 90% of the fats are glycerides of higher fatty acids; like palmitic, stearic, oleic and myristic acids. The remaining 10% consist of lower fatty acids; like caproic, caprylic and butyric acids. Milk also contains some cholesterol, phospholipids and free fatty acids. The major carbohydrate is lactose. Milk is rich in vitamin A and B2. It is the most important source of calcium and phosphorous; but deficient in iron and copper. The mineral composition in milk is as follows (mg%): Quantitative Experiments.

5.18 Experiment No. 18 Determination of Titrable Acidity and Ammonia in Urine

Principle: Maintenance of intracellular pH in the tissues and in the body fluids is vital for normal cellular function. Kidneys help in maintaining the body acid-base balance by increasing or decreasing the secretion of H+ in the urine. The H^+ ions secreted by kidneys are buffered in the tubular fluid by HPO_4^{2-} filtered from glomerule and by NH_3 synthesized and secreted by renal tubular cells. The titrable acidity of urine is mainly due to acid phosphates (H_2PO^{2-} or NaH_2PO_4) and to a less extent, weak organic acids. It can be determined by titrating urine with a standard alkali using phenolphthalein as the indicator.

$$NaH_2PO_4 + NaOH \rightarrow Na_2HPO_4 + H_2O$$

Calcium should be removed as Ca-oxalate before titration as it otherwise interferes by being precipitated as calcium phosphate. Ammonia is synthesized in renal cells by the hydrolysis of glutamine and by trans-deamination as well as oxidative deamination. Ammonia is determined by the formal titration method. In this method, neutralized formaldehyde is added to a solution containing ammonium salts; H^+ ions are liberated and are titrated with a standard alkali. Hexamethylenetetramine $N_4(CH_2)_6$, is other product in this reaction.

$$4NH_4^+ + 6HCHO \rightarrow 4H^+ + N_4(CH_2)_6 + 6H_2O$$

Reagents:

1. 0.1N NaOH;
2. Potassium oxalate powder;
3. Phenolphthalein (0.1% solution in ethanol);
4. Formaline, 20% v/v neutralised solution.

Methods of Determination Titrable Acidity

1. Pipette 25 ml of urine into a 250 ml conical flask and add 2-spatula full potassium oxalate powder to precipitate
2. Add 5 ml of neutralized formalin to the above flask. Pink color disappears due to the liberation of H^+ from NH_4 salts. Titrate the mixture in conical flask with 0.1N NaOH until the pale pink color reappears. Record the second titre value (B ml).

Observation

1. Volume of 0.1 N NaOH required to neutralize titrable acidity (**A ml**).
2. Volume of 0.1 N NaOH required for formol titration (**B ml**).

Calculations

1. Titrable acidity: Volume of 0.1 N NaOH required to neutralize the titrable acidity in 25 ml urine = **A** ml

- Volume of 0.1 N NaOH required for 100 ml urine = A × 4 ml
- Titrable acidity of 100 ml urine = **4A** ml of 0.1 N NaOH.

Assuming 24h urine output 1500 ml, titrable acidity of urine = **4A × 15** ml/day.

2. Total ammonia: calcium. Add 2 drops of phenolphthalein mix and titrate with 0.1N NaOH from a burette. Note the titre value (**A** ml) when a permanent pale pink color appears. Preserve the contents for 'ammonia' estimation.

Volume of 0.1 N NaOH required for 100 ml urine = **B × 4** ml

Since 1 ml 0.1 N NaOH \ 1.7 mg of NH_3

- Ammonia content of 100 ml urine = 4B × 1.7 mg
- Ammonia content of urine = 4B × 1.7 × 15 mg/day.

Clinical Significance

Titrable acidity of urine amounts to 200 to 300 ml/day; urinary ammonia amounts 0.5 to 0.85 g/day. Their values rise on starvation, diabetic ketosis and acidosis. Titrable acidity and ammonia are decreased in alkalosis.

4. Determination of Chlorides in Urine

Principle: When a known volume of urine is acidified with HNO_3, chloride is precipitated as AgCl by adding a measured excess of standard $AgNO_3$ solution. The amount of $AgNO_3$ left unused after the precipitation is determined by titrating with standard ammonium or potassium thiocyanate solution, using ferric ammonium sulphate to indicate the end point. Silver with thiocyanate form silver thiocyanate. Excess thiocyanate gives a salmon red color due to formation of ferric thiocyanate by reacting with ferric ammonium sulphate (ferric alum).

1. $AgNO_3 + NaCl \rightarrow AgCl + NaNO_3$
2. $AgNO_3 + NH_4CNS \rightarrow AgCNS + NH_4NO_3$
3. $6\ NH_4CNS + (NH_4)_2SO4,\ Fe_2(SO_4)_3 \rightarrow 2Fe(CNS)3 + 4(NH_4)2SO_4$

Reagents

1. Standard 0.17 N $AgNO_3$ solution: Dissolve 29.061 g $AgNO_3$ in distilled water and volume make up to 1 litre. 1 ml of this solution is equivalent to 10 mg NaCl or 6 mg Cl^-.
2. Standard 0.17 N ammonium thiocyanate (NH_4CNS) solution: 13 g NH_4CNS in distilled water and volume make up to 1 litre. Now take 20 ml of standard $AgNO_3$ solution, 4 ml conc. HNO_3 and 5 ml ferric alum solution in a flask, diute to 100 ml with distilled water and titrate with NH_4CNS solution. Dilute the NH_4CNS solution with distilled water to make 1 ml of that solution is exactly equivalent to 1 ml of 0.17 N $AgNO_3$ solution. Standard 0.17 N potassium thiocyanate (KCNS) solution can also be used: Use 16.6 g KCNS instead of 13 g NH_4CNS.

3. Saturated solution of ferric alum (Ferric ammonium sulphate): $(NH_4)2SO_4$, $Fe_2(SO_4)_3.24H_2O$.

4. d. Conc. HNO_3

Method

In a 25 ml conical flask, take 5 ml of urine, 2 ml conc. HNO_3 (to prevent urate precipitate), 10 ml $AgNO_3$ (swirl the solution to mix) and 10 drops ferric alum. Titrate this mixture with thiocyanate. End point: Persistent salmon pink color for 30 second. If the first drop of thiocyanate solution gives the color, indicates the concentration of chloride in urine is high. In that case, repeat the whole method using 20 ml $AgNO_3$. Do not use tap water for washing as it may contain chloride as impurities. The end point color must persist for 30 seconds.

Calculation

Titre value for 5 ml urine ≅ A ml.

Volume of $AgNO_3$ which reacted with chloride in urine @ (10-A) ml

Since, 1 ml $AgNO_3$ ≅ 10 mg NaCl or 6 mg Cl^-

Thus, (10-A) ml $AgNO_3$ = 10 × (10-A) mg NaCl or 6 × (10-A) mg Cl^-

5 ml urine ≅10 × (10-A) mg NaCl or 6 × (10-A) mg Cl^-

100 ml urine ≅ 10 × (10-A) × 20 mg NaCl or 6 × (10-A) × 20 mg Cl^-

Clinical Significance: On an average diet, 8–15 g (or 170–250 mEq) of chloride is excreted per day. Normal serum level of chloride is 96–106 mEq/L. Vomiting and diarrhea result in low serum chloride levels and a consequent fall in urinary chlorides. When the serum level is much below 103 mEq of chloride/ litre, the urinary excretion of chloride is low.

5. Determination of Glucose in Urine

Principle: Benedict's quantitative reagent, which is $CuSO_4$ in alkaline medium, is reduced by glucose in urine to cuprous oxide, and combines with KCNS in the solution to form white ppt of cuprous thiocyanate preventing precipitation of Cu_2O as red ppt. On complete reduction, the blue color completely disappears to give a green color, which quickly changes to colorless with an additional drop of urine. The reaction is as follows:

1. $CuSO_4 + Na_2CO_3 + H_2O \rightarrow Cu(OH)_2 \rightarrow CuO$ (black) (Prevented by Na-citrate)
2. Cu^{2+} + Glucose → Cu_2O (red) (Prevented by potassium ferricyanide)
3. $Cu^{2+} + KCNS \rightarrow CuCNS + K^+$

Note: Na_2CO_3 neutralizes any urinary acidity and also liberates CO_2 during titration, which is to an extent useful in preventing reoxidation of cuprous ions.

Reagents: Benedict's qualitative reagent, Benedict's quantitative reagent and Na_2CO_3 powder.

Method: Pipette exactly 20 ml of Benedict's quantitative reagent in a conical flask. Add about 5 gm Na_2CO_3 powder and a few pieces of glass beads to prevent bumping of the solution on boiling. Boil the solution on a low oxidizing flame. Add the diluted urine initially at the rate 0.5 to 1 ml at a time, wait for a few seconds to complete the reaction; and continue with titration.

End point: Complete discharge of blue color; very faint green or white ppt.

Note: Boiling should be uninterrupted and gentle to avoid bumping.

Calculation

1 ml of Benedict's quantitative reagent is reduced by 2 mg of glucose.

So, 20 ml Benedict's reagent is reduced by 40 mg glucose.

A ml (burette reading) diluted urine \ 20 ml Benedict's reagent @ 40 mg glucose. Or, A/D ml of undiluted urine @ 40 mg glucose.

100 ml of undiluted urine @ 40 × D/A × 100 mg glucose @ 4 × D/A g glucose.

Clinical significance: Glucose in urine is generally associated with diabetes mellitus.

6. Reagent preparation

1. **0.005 M Sodium thiosulfate ($Na_2S_2O_3$):** Dissolve 1.24g **$Na_2S_2O_3$.**$5H_2O$ in 1000 ml water. Store in a cool, dark place.
2. **2N Sulfuric acid (H_2SO_4):** Slowly add 6 ml concentrated H_2SO4 to 90 ml water. Make to 100 ml with water. The solution is stable indefinitely. Always add acid to water, not water to acid,
3. **10% Potassium iodide (KI):** Dissolve 100 g KI in 1000 ml water. Protect reagent from direct light. Store in an amber/brown bottle and in a cool, dark place.
4. **Starch indicator solution:** 1 g chemical starch in 10 ml double-distilled water. Continue to boil until it completely dissolves. Add the saturated NaCl solution to make 100 ml starch solution.
5. **Methyl orange indicator:** Dissolve 0.0l g methyl orange in l00 ml water.
6. **Bromine water:** Place 5 ml in a small flask neutralize bromine water of its halogen component by sprinkling sodium metabisulfite, sodium sulfite.
7. **Sodium sulfite solution:** Dissolve 1 g sodium sulfite in l00 ml Water freshly.
8. **Preparation of 0.02 N Potassium Dichromate Standard Solution:** preparation 100ml of 0.02 N solution: M.wt. $K_2Cr_2O_7$ = 294,187 g
9. **Acetate buffer, 0.2 M, pH 4.7:** Dissolve 27.2 g sodium acetate crystals in about 500 ml distilled water. Adjust pH to 4.7 with glacial acetic acid and make up to 1L with water.
10. **Acetate buffer, 3 M, pH 5.0:** Dissolve 40.8 g sodium acetate crystals in about 70ml distilled water. Add 8 ml glacial acetic acid and make up the volume to 100ml with water. This buffer is used for osazone preparation.
11. **Alkaline copper reagent:** Dissolve 40 g anhydrous sodium carbonate in about 400 ml distilled water and transfer it to a liter flask. Dissolve 7.5 g tartaric acid in this solution and add 4.5 g copper sulphate. Mix and make the volume to 1litre.
12. **Ammonium molybdate [(NH4)Mo7O24, 4H2O] reagent:** Dissolve 100

g molybdic acid in 144 ml of NH_4OH (sp. Gr. 0.9) and 271 ml water. Add this solution with constant stirring slowly into 489 ml conc. HNO_3 and 1148 ml water. Keep this mixture for several days and then filter.

13. **Antimony trichloride ($SbCl_3$) reagent:** 25% SbCl3 in chloroform.

14. **Barfoed's reagent:** Dissolve 25 g copper acetate in 450 ml boiling water. A precipitate may be formed. Add immediately 4 ml glacial acetic acid to the hot solution, stir to mix. Cool and make up to 500 ml with water. Filter off any impurity's sediment.

15. **Benedict's qualitative reagent:** Dissolve with the aid of heat, 173 g of sodium citrate and 100 g of anhydrate sodium carbonate in about 100 ml water in a beaker. Separately dissolve 17.3 g $CuSO_4$, $5H_2O$ in about 100 ml water and transfer this to the first solution, slowly with stirring. Make up the volume to 1L.

16. **Benedict's quantitative reagent:** Dissolve 200 g of sodium citrate, 75 g anhydrate sodium carbonate, 125 g potassium thiocyanate in about 600 ml water with the aid of heating. Separately dissolve 18 gm $CuSO_4$, $5H_2O$in about 100 ml water and transfer this to the first solution, slowly with stirring. Add 5 ml of 5% potassium ferrocyanide. Make up the volume to 1L.

17. **Luca's reagent:** Take 135g of anhydrous zinc chloride and dissolved in 100ml of concentrated hydrochloric acid.

18. **Iodine solution:** Dissolve 5g of potassium iodide in 40ml of water. To that solution add 1g of solid iodine and dissolved it completely.

19. **Tollen's reagent:** Take 5mL of $AgNO_3$ solution in a clean test tube and add 2 drops of 10% NaOH solution. A grey ppt. is formed. To this add NH4OH drop wise until the grey precipitate dissolves. This is known as Tollen's reagent.

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