Subject Name: PHARMACEUTICAL INORGANIC CHEMISTRY
Subject Code: BP104TP

Scope: This subject deals with the monographs of inorganic drugs and pharmaceuticals

Objectives: Upon completion of course student shall be able to

1. know the sources of impurities and methods to determine the impurities in inorganic drugs and pharmaceuticals
2. understand the medicinal and pharmaceutical importance of inorganic compounds

Sr No	Course Contents	Total Hrs
1	**Impurities in pharmaceutical substances:** History of Pharmacopoeia, Sources and types of impurities, principle involved in the limit test for Chloride, Sulphate, Iron, Arsenic, Lead and Heavy metals, modified limit test for Chloride and Sulphate **General methods of preparation**, assay for the compounds superscripted with **asterisk (*)**, properties and medicinal uses of inorganic compounds belonging to the following classes	10
2	**Acids, Bases and Buffers:** Buffer equations and buffer capacity in general, buffers in pharmaceutical systems, preparation, stability, buffered isotonic solutions, measurements of tonicity, calculations and methods of adjusting isotonicity. **Major extra and intracellular electrolytes**: Functions of major physiological ions, Electrolytes used in the replacement therapy: Sodium chloride*, Potassium chloride, Calcium gluconate* and Oral Rehydration Salt (ORS), Physiological acid base balance. **Dental products**: Dentifrices, role of fluoride in the treatment of dental caries, Desensitizing agents, Calcium carbonate, Sodium fluoride, and Zinc eugenol cement.	10

3	**Gastrointestinal agents** **Acidifiers:** Ammonium chloride* and Dil. HCl **Antacid:** Ideal properties of antacids, combinations of antacids, Sodium Bicarbonate*, Aluminum hydroxide gel, Magnesium hydroxide mixture **Cathartics:** Magnesium sulphate, Sodium orthophosphate, Kaolin and Bentonite **Antimicrobials**: Mechanism, classification, Potassium permanganate, Boric acid, Hydrogen peroxide*, Chlorinated lime*, Iodine and its preparations	10
4	**Miscellaneous compounds** **Expectorants:** Potassium iodide, Ammonium chloride*. **Emetics**: Copper sulphate*, Sodium potassium tartarate **Haematinics:** Ferrous sulphate*, Ferrous gluconate **Poison and Antidote:** Sodium thiosulphate*, Activated charcoal, Sodium nitrite333 **Astringents**: Zinc Sulphate, Potash Alum	8
5	**Radiopharmaceuticals**: Radio activity, Measurement of radioactivity, Properties of α, β, γ radiations, Half life, radio isotopes and study of radio isotopes - Sodium iodide I131, Storage conditions, precautions & pharmaceutical application of radioactive substances.	7

Impurities In Pharmaceutical Substances

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CONTENTS

- Introduction of Inorganic Chemistry
- History of Pharmacopeia
- Introduction and Classification of Impurities
- Sources of Impurities
- Introduction on Limit test
- Limit test for Chloride, Sulphate, Iron, Arsenic, Lead and Heavy metals
- Modified Limit test for Chloride, Sulphate Application

❖ INTRODUCTION:

- Inorganic Chemistry is the study of the Synthesis, Reactions, Structures and Properties of all the elements and their Compounds except Carbon and its Compounds.
- Inorganic Chemistry deals with the Compounds or molecules which lack carbon atom.
- Pharmaceutical Inorganic Chemistry is a science that makes use of the law of chemistry to study inorganic substances as drugs i.e. their Preparation, Chemical nature, Composition, structure, Influence on an Organism etc.
- Study of Pharmaceutical application of Inorganic Compounds led to the establishment of a new avenue called Pharmaceutical Inorganic chemistry, " which deals with the study of both non-essential and essential elements, their preparation, standard of Purity, test for identification, limit tests to be performed for determining the quality, extent of purity, different formulations, their storage condition and therapeutics uses"


PHARMACOPOEIA
USP
NF
INDIAN
PHARMACOPOEIA
2007
Addendum 2008
INDIAN
PHARMACOPOEIA
1996
British
Pharmacopoeia
JP
Japanese
Pharmacopoeia

❖ INTRODUCTION TO PHARMACOPOEIA:

- "Pharmacopeia is derived from the **Greek words 'Pharmacon' meaning drug or medicine and poeia mean 'to make'**
- The book containing the Standards for Drugs and other related substance are known as Pharmacopoeia and formularies- **Collectively these books are known as the drug Compendia.**

❖ **Classification of Compendia: 1) Official Compendia 2) Non-Official Compendia**

1) **Official Compendia:** Official Compendia are the Complication of Drugs and other related substance which are recognized as legal standard for purity, Quality and strength by a Government agency of respective countries of the origin. E.g. British Pharmacopoeia, Indian Pharmacopoeia, United State Pharmacopoeia, British Pharmaceutical Codex, National Formulary.
2) **Non-Official Compendia:** The books other than official drug Compendia which are used as Secondary reference sources of drugs and other related substance are known as non-official Compendia. E.g. Merck Index, United States Dispensatory.

- The Pharmacopoeia or Formularies Contain a list of drugs and other related Substances regarding their sources, Descriptions, Standard test, Formula for preparing the action and uses, dose, storage condition etc.
- Theses books are prepared under the authority of the Government of the respective Countries.

❖ HISTORY OF PHARMACOPOEIA: (Que: Write a Note on History of Pharmacopoeia)

- The term Pharmacopeia first appears as a distinct title in a work published in Basel, Switzerland in 1561 by Dr. A. Foes but does not appear to have come into general use until the beginning of the 17th Century.
- Today's Pharmacopeia focus mainly on assurance of the quality by various tools of analytical sciences.
- On 15th December 1820, the first United state Pharmacopeia was released. In 1864, the First British Pharmacopeia was published with inclusion of Monograph on Benzoic acid, Gallic acid, Tartaric acid, Camphor, Lactose, Sucrose and Seven alkaloids along with their salts.

1) INDIAN PHARMACOPEIA:

- Indian Pharmacopeia is an official document meant for overall Quality Control and Assurance of pharmaceutical Products marketed in India, by way of Contributing on their safety, efficacy and affordability.
- IP Contains a collection of Authoritative procedures of analysis and specification of drugs.
- IP Prescribed standard for identity, purity and Strength of drug essentially required from health care perspective of human being and animals.
- IP Standard are authoritative in nature.
- The Historical development of pharmacopeia in India traces back to 1563 and the Credit goes to Garcia aorta a Portuguese physician cum teacher.
- The Indian Pharmacopeial List, Published in 1946 formed the seeding for the true official Indian Pharmacopoeia Published in 1955.
- The first edition of Indian Pharmacopeia was published in 1955 but actually the process was started as early as 1944. In 1944 Government of India asked the Drugs Technical Advisory Boards to prepare the list of drug used in having sufficient medicinal value to justify their inclusion in official Pharmacopeia.

❖ Pharmacopeial Description/ Presentation:

- Most of the Pharmacopoeias including Indian Pharmacopoeia Consist of the three major section namely:

  (a) Introduction Including general Notices

  (b) Monographs of the official drugs

  (c) Appendices and Index

(A) Introduction: It is useful pointer to Pharmaceutical progress since last edition.

- It Summarizes the different changes including additions/ deletions in the current edition compared to last edition.

(B) Monographs: The written study of a subject was implied by the word “monograph”.

- These are considered as very important because medicinal substances are used for the cure and prevention of diseases.

- The General Monograph for dosage forms of Active Pharmaceutical Ingredients are grouped together at the beginning of volume II of IP 2010.
- They are followed by the monograph for the APIs, Pharmaceutical aids and Individual dosage forms all in alphabetical order.
- Monograph for other articles of a special nature such as vaccines and Immunosera for human use, herbs and herbal products, blood and blood related products, biotechnology products, veterinary products are given in separate section in Volume III of TP 2010.
- The Pharmacopeial Monograph Contain Following information:

1. **Main Title:** The name of the Substance
2. **Synonym:** The Common name of the substance.
3. **Chemical Formula and Molecular weight of the Substance**: IUPAC Name and Chemical Structure also given.
4. **Category:** Indicates the use of the drug in medicine and pharmaceutical practice. E.g. Antibacterial, antimalarial, diuretics, emetic, expectorant etc**.**
5. **Dose:** Represents the average range of quantities suitable for adults.

6. **Description:** This includes the general physical properties i.e. whether the substance is a solid or liquid, colorless or colored, Crystalline or amorphous, its taste etc.
7. **Solubility:**
8. **Standards:** Prescribes the Standard of purity and Strength e.g. Sodium Bicarbonate IP Contain not less than 99 % and note more than 100.5% Sodium Bicarbonate.
9. **Identification:** This includes some specific and some non-specific tests for Identity of Substance.
10. **Tests of Purity:** These Tests including Melting point, Boiling point, Specific optical rotation, Loss on drying, pH of solution etc. may be applicable for the substance.
11. **Method of Assay:** The term "Assay" is used in pharmacopoeias for quantitative determination of principal ingredients of the official substance and of their preparation.
12. **Storage**: Prescribes some Conditions for the storage of some official substances which are likely to deteriorate if not properly stored.

(C) Appendices and Index: Appendices should include Infra-red spectra of drug, Apparatus for tests and assays, Biological tests and determinations, Chemical tests and assays , Chromatography and electrophoresis etc.

❖ IMPURITIES: (Que: Define Impurities and Classification of Impurities)

- Impurities in pharmaceuticals are the unwanted Chemicals that remain with the Active pharmaceutical ingredients (APIs), develop during formulated APIs to medicines and upon ageing of API/drug Product.
- The presence of these unwanted chemicals even in trace amount may influence the efficacy and safety of the pharmaceutical Product.
- The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of Impurities.
- According to ICH, an impurity in a drug substance is defined as “any component of the new drug substance that is not the Chemical entity defined as the new drug substance.”
- “In the pharmaceutical world an impurity is considered as any other organic material besides the drug substance or ingredients arise out of synthesis and unwanted chemicals that remains with API’s.

❖ TYPES or CLASSIFICATION OF IMPURITIES:

(A) According to ICH Guidelines Classify:

1) Organic Impurities (Process and Drug related)

2) Inorganic Impurities

3) Residual Solvents

(B) According to United States Pharmacopoeia Classify:

1) Impurities in official Articles

2) Ordinary Impurities

3) Organic Volatile Impurities

1) Organic Impurities: Organic Impurities may arises during the manufacturing process and storage of the drug substance which includes starting material, by-product, intermediate, degradation product, reagent, ligands, catalysts and enantiomeric Impurity.

- These are the actual and Potential Impurities most likely to arise during the synthesis, Purification and Storage of the Drug Substance.

2) **Inorganic Impurities:** Inorganic Impurities derived from the manufacturing Process used for bulk drug and they are normally known and identified.

- Inorganic Impurities involve Reagents, Ligands, Catalysts, heavy metals or other residual metals, inorganic salts, filter aids, charcoal etc.

3) Residual Solvents: Residual Solvent are organic volatile chemical used during the manufacturing process or generated during Production.

- It is very difficult to remove theses solvents completely by work-up process and most common techniques for measuring residual solvent is gas chromatography because of the small size and volatile nature of solvent molecule.

Residual Solvent are classified 1) Class I Solvent : Benzene and Carbon Tetra chloride

2) Class II Solvent: Methylene Chloride, Methanol. Pyridine, Toluene, Acetonitrile are the most Common Solvent used.

3) Class III Solvent: Acetic acid, Acetone, Isopropyl alcohol, Butanol.

- Other Impurities Include like Polymorph Impurities, Genotoxic Impurities, Excipient Impurities, Elemental Impurities, Packaging material induced Impurities.

❖ SOURCES OF IMPURITIES: (Que: Write a S.N on Source of Impurities)

- Impurities are the Substance present in a confined amount either in liquid, gas or solid preparation which differ from the original chemical composition of material or Compound.
- These are either naturally occurring or added during synthesis of a chemical product.
- The type and amount of impurity present in chemicals or Pharmaceutical Substances depends upon several factors such as:

1) Raw Material

2) Method of Manufacturing i) Reagent Employed in manufacturing Process ii) Reagent used to eliminate other Impurities iii) Solvent

3) Reaction Vessel

4) Atmospheric Contamination during the Manufacturing Process.

5) Intermediate products in the manufacturing Process.

6. Manufacturing Hazards i) Contamination From Particulate Matter ii) Cross-Contamination of the Product iii) Contamination by Microbes iv) Error in Manufacturing Process v) Error In Packaging

7. Instability of Product i) Temperature ii) Chemical Instability iii) Changes in Physical Properties.

8. Chemical Processes used in manufacture

9. Methods or the process used in manufacture

10. Defects in the Manufacturing Process

11. Storage Condition and Decomposition of Product during Storage

1) Raw Material:

- Impurities such as arsenic, lead, heavy metal etc. are present in raw material and found in final Product. Therefore become necessary to use pure chemicals and substances as raw materials for the Manufacturing Process.
- Trace of Impurities like lead, copper etc. usually present in Raw material may pass on the final Product via the manufacturing Process.

e.g. Zinc Sulphate is prepared by reacting either zinc oxide or zinc metal with Sulphuric acid.

$$ZnO + H_2SO_4 \longrightarrow ZnSO_4 + H_2O$$

$$Zn + H_2SO_4 \longrightarrow ZnSO_4 + H_2$$

Zinc metal and Zinc Oxide Contain Al, Cu, Mg, Mn, Ni and Fe as Impurities and theses Impurities are likely to be carried in the manufacturing process in appreciable amount to the final Product as $ZnSO_4.7H_2O$.

2. Method Of Manufacturing: Method of Manufacturing may introduce new impurities into the final Product arising due to Contamination by Reagents, Catalysts and Solvent employed.

i) **Reagent Employed in manufacturing Process:** Calcium Carbonate contains soluble alkali as impurity which arises from sodium carbonate employed in the Process.

- Calcium carbonate is prepared by interaction of a soluble calcium salt with a soluble carbonate.
- The Final product Calcium carbonate contain small amount of alkali as impurities which can not be removed by the washing Process.

$$CaCl_2 + Na_2CO_3 \longrightarrow CaCO_3 + 2NaCl$$

Calcium Chloride Sodium Carbonate Calcium Carbonate Sodium Chloride

ii) Reagent used to eliminate other Impurities :

e.g. Preparation of Potassium Bromide Interaction between Potassium carbonate with an iron (III, II) bromide made by treating scrap iron under water with excess bromine.

$$4K_2CO_3 + Fe_3Br_8 \longrightarrow 8KBr + Fe_3O_4 + 4CO$$

- Barium is used in the Preparation of Potassium Bromide to remove Sulphate Impurities which in turn arise from the bromine used in the Process.
- It is likely that potassium bromide will now be contaminated by trace of barium in it.

iii) Solvent : Small amount of solvent employed in preparations of final Product and Purification of reaction intermediate may also found in Contamination of Pharmaceutical Substances.

e.g. Water is Cheapest and can be Major source of Impurity.

1) Tap Water: It Contain Impurities like calcium ion, Magnesium ion, Sodium ion, Chloride ion, Carbonate ion in trace amounts. The use of tap water on large scale can lead to contamination of final Product.

2) **Softened Water**: It is Free from Calcium and Magnesium ion (divalent ion) but contain more sodium and Chloride ion as a impurity. Therefore final Product obtained by using Softened water will contain sodium and Calcium as impurities.

3) **Demineralized Water:** It is prepared by Ion exchange resin and is free from all kind of ion. It may have pyrogens, bacteria and organic impurities.so it better than tap water and softened water but the economic factors discourage its use on large scale.

4) Distilled Water: It is free from all organic and Inorganic Impurities and best solvent but it is quite expensive.

3. Reaction Vessel: The reaction vessel employed in the manufacturing process may be metallic such as copper, galvanized iron, silica and glass.

- Some reagents employed in the Manufacturing Process may react with metal of reaction vessel leading to the traces of metal impurities into the solution and finally contaminating the Product.
- Glass vessels may give traces of alkali to the Solvent.
- E.g. Strong acid leaches out alkali to the Solvent from Borosilicate glass.

4. Atmospheric Contamination during the Manufacturing Process: Atmosphere may contain dust and some gases like Carbon dioxide, Sulphur dioxide, arsenic and hydrogen sulphides.

- These may Contaminate the final product during manufacturing process.
- E.g. NaOH readily absorbs atmospheric carbon dioxide when exposed to atmosphere.

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

Sodium Hydroxide Carbon dioxide Sodium Carbonate Water

5) Intermediate products in the manufacturing Process:

$$6NaOH + 3Br_2 \longrightarrow NaBrO_3 \xrightarrow{\text{Heating with Charcoal}} 5NaBr + 3CO$$

Sodium Hydroxide Bromine Sodium bromate Sodium Bromide Carbon Monoxide

- Sometimes an intermediate substance produced during manufacturing process may contaminate the final product.
- e.g. Sodium bromide is prepared by the reaction of NaOH and Bromine slight excess. The Sodium bromate an intermediate product is reduced to sodium bromide by heating the residue (Obtained by evaporating the Solution to dryness) with Charcoal.

- Sodium Bromate is not Completely converted to NaBr then it is likely to be present as an impurity.

6. Manufacturing Hazards: If Manufacture is to control and check Impurities from all above mentioned Impurities, there exist certain manufacturing hazards which can lead to product Contamination.

i) Contamination from Particulate Matter: The Particulate Contamination Arises from dirty or improperly maintained equipment and also arise from the Bulk material used in the Formulation.

e.g. Contamination of eye ointments packed in metal containers of tin and aluminium.

ii) Cross-Contamination of the Product: It can Occur air-bone dust arising from bulk powders, granules and tablets.

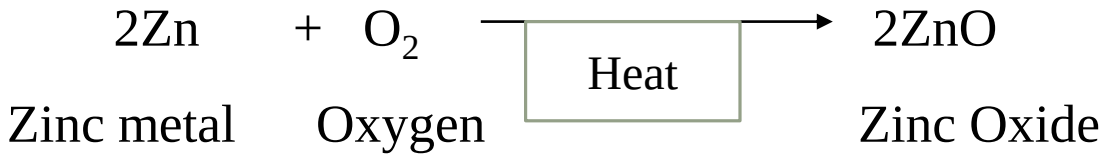
- It is dangerous particularly in case of steroidal and other synthetic hormones therefore it should be carefully Controlled.

iii) Contamination by Microbes: Many Products like liquid preparation and Creams intended for topical application are liable to be contaminated by microbes during manufacturing.

- For Parenteral and Ophthalmic Preparations Sterility testing is done and it Provides an adequate control for Microbial Contamination in such Preparation

iv) Errors in Manufacturing Process: Defects like Incompleteness of reaction, imperfect mixing etc. may results in the Production of impure compound.

e.g. Zinc Oxide is produced by heating metallic zinc in a current of air (Incompleteness of Reaction)



- If Zinc metal is not Completely converted into ZnO, Small amount of Zinc metal may be present in the final product as impurity.

v) Error in Packaging: Similar looking products such as tablet of same size, shape and colour, Packed in similar container can result in mislabelling of either or both the Products.

7. Instability Of Product:

i) Temperature: The rate of Chemical decomposition and physical changes of stored product depends upon Temp.

- The Susceptible substances may have assigned storage Temp. Requirements and protect against undesirable decomposition.

ii) Chemical Instability: Many Pharmaceutical Substance undergo chemical decomposition when storage conditions are inadequate.

- This Chemical decomposition is catalysed by light, traces of acid or alkali, traces of metallic impurities, air oxidation, carbon dioxide and water vapours.

iii) Changes in Physical Properties: Pharmaceuticals my undergoes changes in Physical properties during changes.

- There can be change in crystal size and size. These Physical Changes may results in significant changes in physical appearance, Pharmaceutical and therapeutic effect of the Product.

❖ Limit Test:

Limit : A value or amount that is likely to be present in a substance.

Test: to examine or to investigate

Impurities: a Foreign matter (Unwanted Chemicals) Present in a Compound.

- Limit test is defined as quantitative or Semi-quantitative test designed to identify and control small quantities of impurity which is likely to be present in the substance.
- Limit test is generally carried out to determine the inorganic impurities present in the Compound.
- Limit test is nothing but to identify the impurities present in the substance and compare it with the standard.

Importance of Impurities:

1. To fine out the harmful amount of Impurities
2. To find out permissible/impermissible amount of Impurities.

❖ **<u>Limit Test for Chloride</u>**: (Que: Write a S.N on Limit test for Chloride)

❖ **Principle:**

➢ The limit test for chloride has been based on the simple reaction between Silver nitrate and Soluble Chloride (NaCl) in Presence of dilute nitric acid to obtained Silver Chloride.

➢ The Silver Chloride produced in the presence of dilute nitric acid makes the test solution turbid and turbidity depend upon the amount of Chloride present in the Substance.

❖ **Reaction:**

$$NaCl + AgNO_3 \xrightarrow{HNO_3} AgCl\downarrow + NaNO_3$$

Sodium Chloride	Silver Nitrate		Silver Chloride (White Curdy ppt. or White turbidity)		Sodium Nitrate

❖ **Method for the Limit test for Chloride:**

❖ **Procedure:**

Test Solution	Standard Solution
Specified substance (1 gm) + 10 ml of Water take in Nessler Cylinder A	1 ml of 0.05845 % w/v solution of Sodium Chloride + 10 ml Water in Nessler Cylinder B
↓	↓
Add 1 ml of HNO_3 and make up the volume With 50 ml water + 1 ml of silver nitrate add, stir With glass rod and set aside for 5 min on white Background.	Add 1 ml of Dil. HNO_3 and make up the volume With 50 ml water + 1 ml of silver nitrate add, stir With glass rod and set aside for 5 min on white Background.
↓	↓
Observe Opalescence/Turbidity	Observe Opalescence/Turbidity

- **Observation**: Turbidity (Amount of chloride) present in the test solution is compared with a Turbidity of Standard Solution produced by addition of silver nitrate to a standard having known amount of chloride and same volume of nitric acid as used in test solution.
- **Conclusion:** If the turbidity from the sample has been less than the standard turbidity the sample will pass the limit test.
- If the turbidity from the sample has been more than the standard turbidity the sample will fail the limit test.

Reagent Preparation:

1. Dil. Nitric acid: 106 ml Nitric acid is diluted to 1000 ml water
2. Silver nitrate solution (5%) : 5 gm of Silver nitrate is dissolved in 100 ml of water
3. Standard solution of NaCl solution: Dissolve 0.05845 gm of NaCl in 100 ml distilled water.

https://www.youtube.com/watch?v=FbRLLdbbjFQ (Watch video for Limit test of Chloride using think link)

❖ Modified Chloride limit test:

➢ With reference to International Pharmacopoeia 6th edition 2016, the limit test of chloride has been modified in the context of standard solution preparation. Earlier the standard solution of chloride was prepared by dissolving NaCl but now it has been modified by using HCl instead of NaCl.

❖ **Limit Test for Sulphate**: (Que: Write a S.N on Limit test for Sulphate)

❖ **Principle:**

➢ The Principle involved in the limit test of sulphate is precipitation method and comparison of the turbidity produced by test solution with standard solution containing a known amount of Sulphates.

➢ The Limit test for sulphate is carried out on the basis of the reaction between barium chloride and soluble sulphate in presence of dilute HCl so the Formation of barium sulphate as a precipitation form.

❖ Reaction:

- Due to the formation of Precipitates in the solution appears turbid and the extent of turbidity depends on the amount of sulphates present.
- Barium chloride test solution in the IP has been replaced by Barium Sulphate reagent which is having Barium chloride, Sulphate free alcohol and a Solution of Potassium sulphate.
- Potassium sulphate has been added to increase the sensitivity of the test.
- Barium chloride has been produced barium ion react with sulphate ion to form turbidity of barium sulphate.
- Barium Sulphate Present in the reagent acts as seeding agent for precipitation of barium sulphate.
- Alcohols helps to prevent super-saturation and thus produce a more uniform Turbidity.

❖ **Method for the Limit test for Sulphate:**

❖ **Procedure:**

Test Solution	**Standard Solution**
Specified substance (1 gm) + 2 ml Dil. HCl + to 45 ml Water take in Nessler Cylinder A	1 ml of 0.1089 % w/v solution of K_2SO_4 + 2 ml Dil. HCl + to 45 ml Water take in Nessler Cylinder B
↓	↓
Add 5 ml of $BaSO_4$ reagent and stir with glass Rod and set aside for 5 min on white Background.	Add 5 ml of $BaSO_4$ reagent and stir with glass Rod and set aside for 5 min on white Background
↓	↓
Observe Opalescence/Turbidity	Observe Opalescence/Turbidity

- **Observation**: Turbidity (Amount of Sulphate) present in the test solution is compared with a Turbidity of Standard Solution produced by addition of Barium Sulphate Reagent to a standard having known amount of Sulphate and same volume of HCl as used in test solution.
- **Conclusion:** If the turbidity from the sample has been less than the standard turbidity the sample will pass the limit test.
- If the turbidity from the sample has been more than the standard turbidity the sample will fail the limit test.

Reagent Preparation:

1. **Barium Sulphate Reagent:** Dissolve 12 gm of $BaCl_{2.}$ $2H_2O$ in 1000 ml water to make 0.05 M barium chloride solution. To 15 ml of the prepared solution add 55 ml water, 20 ml alcohol, 5 ml of 0.0181% w/v Potassium sulphate solution and make up the volume upto 100 ml.
2. **Standard Potassium sulphate solution:** Accurately weigh 0.1089 gm of Potassium sulphate in 100 ml water.

❖ Modified Sulphate limit test:

- From IP 1996 onwards, Limit test for sulphate has been modified to a great extent. It has done away the requirement of Barium Sulphate reagent. However it still uses alcohol along with Barium Chloride to produce Comparable Turbidity.
- https://www.youtube.com/watch?v=FAkbYY9Y_vQ (Watch video for Limit test of Sulphate using think link)

❖ Limit Test for Iron: (Que: Write a S.N on Limit test for Iron)

❖ Principle:

- The Limit test for iron is based on the reaction of iron in Ammonical solution in presence of citric acid with thioglycollic acid when a pale pink to deep reddish purple colour is formed due to ferrous compound.
- This test is based on the formation of purple colour by reaction of iron with thioglycollic acid to form ferrous thioglycollate in the presence of ammonium citrate.
- Iron Present as an impurity in the sample react with thioglycollic acid to form ferrous thioglycollate which is responsible for the appearance of purple colour in the solution.

- This reaction occur if the given sample contains iron as an impurity even in trace amount.
- The Intensity of colour produced by Test Solution is compared with the standard solution (Ferric ammonium sulphate) by viewing vertically.
- If the colour from test solution is less dark than the standard then the sample passes the test.
- The colour due to ferrous compound gets destroyed by oxidising agents and alkalies. The state of Oxidation of iron has been immaterial as iron (III) Fe^{+2} gets reduced to iron (II) Fe^{+3} by thioglycollic acid.

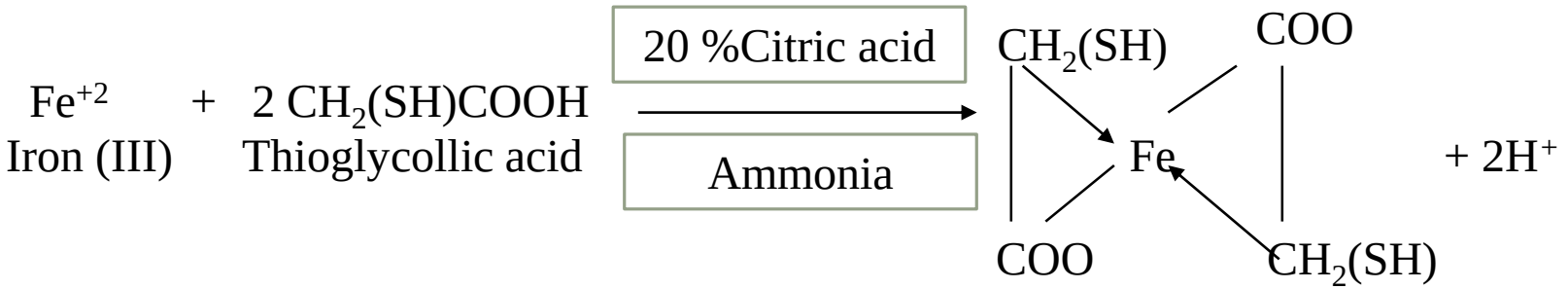
❖ Reaction:

$$CH_2Cl.COOH + KSH \longrightarrow CH_2(SH)COOH + KCl$$

Monochloroacetic acid — Potassium hydrogen sulphide — Thioglycollic acid

- Earlier ammonium thiocyanate reagent was used for the limit test of iron. Since, Thioglycollic acid is a more sensitive reagent for iron, it has replaced ammonium thiocyanate in the test.

- It is believed that the thioglycollic acid test has been more sensitive than the ammonium thiocyanate test.



Ferrous Thioglycollate (Chelate)

- Ferrous Thioglycollate is colourless in acid or neutral solution. In acidic media Fe^{+2} is oxidized to Fe^{+3} in presence of thioglycollic acid. Hence, the solution become colourless. The Purple colour developed only in the presence of alkali.

❖ **Reagent Preparation:**

1) **Preparation of Standard solution of Iron:** It is Prepared by adding 0.173 g of Ferric ammonium sulphate 10 1.5 ml of HCl and adding sufficient water to produce 1000 ml. Each ml of solution contains 0.02 mg of iron.
2) **Citric acid (20%):** Dissolve 20 gm of iron free citric acid in 100 ml of Distilled water.
3) **Ammonia Solution (10%):** Dissolve 10 ml of Ammonia in 100 ml of Distilled water.

❖ Method for the Limit test for Iron:

❖ Procedure:

TEST SOLUTION	STANDARD SOLUTION	REASONS
Dissolve 1 gm of the Sample in 40ml of water in Nessler's Cylinder A.	2 ml Standard Solution of iron Solution in 40 ml of water in Nessler's Cylinder B.	The Aqueous Solution will leach out all the iron present in the sample or test.
Add 2 ml of 20% Iron free Citric acid.	Add 2 ml of 20% Iron free Citric acid.	Citric acid Prevents the Precipitation of Iron with ammonia and keep Iron free in solution for Combine with Thioglycollic acid.
Add 0.1 ml of Thioglycollic acid	Add 0.1 ml of Thioglycollic acid.	Thioglycollic acid acts as a oxidising agent its convert Ferric ions to ferrous ions and ferrous ions react with Thioglycollic acid to from Ferrous Thioglycollate.
Make this solution alkaline with iron free ammonia solution.	Make this solution alkaline with iron free ammonia solution.	The Ferrous thioglycollate formed is colourless in medium but in alkaline medium it give purple colour,
Dilute to 50 ml mark with Distilled water	Dilute to 50 ml mark with Distilled water	Equal Volumes of test and standard are compared easily.

- Comparison done by viewed Vertically**.**

❖ **Observation**: The intensity of coloured produced by test solution is compared with the standard solution obtained by taking standard iron solution containing 0.04 mg of iron.

❖ **Conclusion:** If the Intensity of Purple Colour in sample has been less than the standard the sample will pass the limit test.

- If the Intensity of Purple Colour in sample has been more than the standard the sample will fail the limit test.
- https://www.youtube.com/watch?v=r98cjAmzNCY&pbjreload=101 (Watch video for Limit test of Iron using think link)

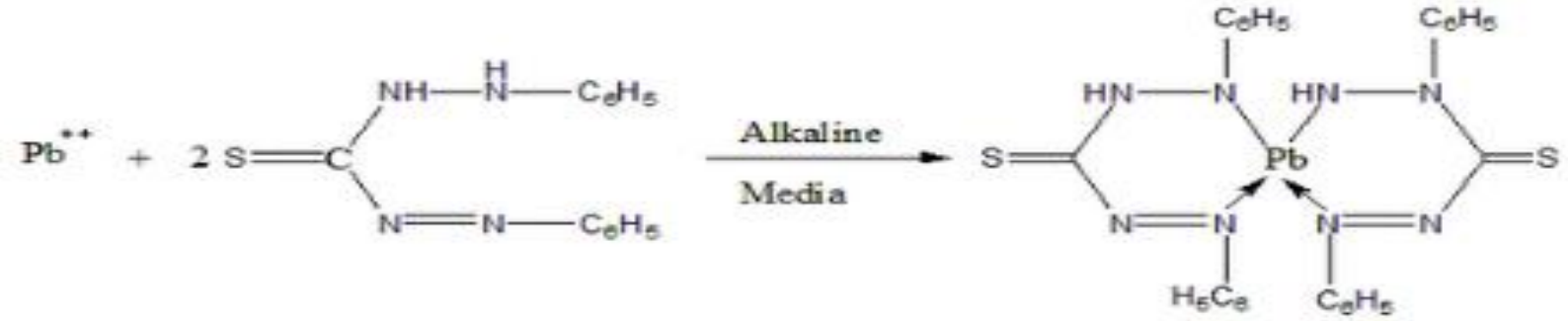
❖ Limit Test for Lead: (Que: Write a S.N on Limit test for Lead)

- Lead is a most undesirable impurity in medical compound and come through use of sulphuric acid, lead lined apparatus and glass bottles used for storage of chemicals.
- Common Sources of lead impurities are:

1. Equipment used for manufacturing
2. Storage Container
3. From Packaging material

4. Principle:

- Limit test of lead is based on the reaction of lead and diphenylthiocarbazone (dithizone) in alkaline solution to form lead dithizonate complex which is violet in colour.



- Lead Present as an impurity in the sample reacts with dithizone to form lead thiozonate which is responsible for the appearance of violet colour in the solution.
- This reaction will only occur if the given sample contains lead as an impurity even in trace amount.
- In this test, Nessler Cylinders are not used instead it is performed by extraction with the help of separating funnel.
- The original colour of dithizone in chloroform is green while the lead dithizonate complex is violet in colour. The intensity of the violet colour of the complex depending upon the quantity of lead present in the solution is compared with that of the standard colour produced by treating standard solution containing definite amount of lead in the similar manner.
- Intensity of this violet colour is compared in both the tests and standard solution in chloroform solvent medium.

❖ Reagent Preparation:

❖ **Preparation of standard lead solution (1ppm Pb):** Dissolve 0.400 gm of lead nitrate in water containing 2 ml of dilute nitric acid and add sufficient water to produce 250 ml This gives standard lead solution (1% Pb). Standard lead solution (1 ppm Pb) is prepared by diluting 1 volume of standard lead solution to 1000 ml with water.

- **Preparation of dithizone extraction Solution:** Dissolve 30 mg of dithizone in 1000 ml Chloroform and add 5 ml of 95% Methanol. The solution is stored in refrigerator. Before use the solution is shaken with about half of its volume of 1% v/v nitric acid solution and the acid is discarded.
- **Preparation of dithizone standard solution:** Dissolve 10 mg of dithizone in 1000 ml of Chloroform.

Method for the Limit test for Lead:

- **Procedure:**

TEST SOLUTION	STANDARD SOLUTION	REASON
4 gm of Substance (Lead Nitrate) dissolve in 20 ml of dilute Nitric acid.	Take a 4 ml standard lead solution.	Suspected sample is taken which might have an impurity of Lead.
Add 6 ml of Ammonium Citrate.	Add 6 ml of Ammonium Citrate.	Ammonium Citrate is added to maintain optimum pH and to prevent the formation of undesirable Precipitates.
Add 2 ml of Potassium Cyanide and 2 ml of hydroxylamine HCl	Add 2 ml of Potassium Cyanide and 2 ml of hydroxylamine HCl	Cyanide forms Complexes with all the interfering metals in the solution.

TEST SOLUTION	STANDARD SOLUTION	REASON
Add 2 drops of Phenol red.	Add 2 drops of Phenol red.	Used as an Indicator to develop colour at the end of the process.
Make the solution alkaline by adding ammonia solution.	Make the solution alkaline by adding ammonia solution.	Ammonia provide basic Conditions to develop red Colour.
Extract with 5 ml of dithizone until it become green.	Extract with 5 ml of dithizone until it become green.	At least 2-3 extraction are done is separating funnel and mix all the extract in the end.
Combine dithizone extract are shaken for 30 min with 30 ml of Nitric acid and chloroform layer is discarded.	Combine dithizone extract are shaken for 30 min with 30 ml of Nitric acid and chloroform layer is discarded.	Acidic layer (Contain free lead Pb^{+2}) is separated from Chloroform. Nitric acid make lead free from Chloroform layer.
To the acid solution add 5 ml of Standard dithizone solution.	To the acid solution add 5 ml of Standard dithizone solution.	Violet colour appears due to Complex lead-dithizonate.
Add the 4 ml of Ammonium cyanide	Add the 4 ml of Ammonium cyanide	Maintains Optimum pH.
Shake for 30 min. add Observe the colour.	Shake for 30 min. add Observe the colour.	Comparison of Colour.

❖ **Observation**: The intensity of coloured produced by test solution is compared with the standard solution.

❖ **Conclusion:** If the Intensity of Violet Colour in sample has been less than the standard the sample will pass the limit test.

➢ If the Intensity of Violet Colour in sample has been more than the standard the sample will fail the limit test.

❖ Limit Test for Heavy Metal: (Que: Write a S.N on Limit test for Heavy Metal)

- The Limit test for heavy metals is designed to determine the content of metallic impurities that are coloured by Hydrogen Sulphide or Sodium Sulphide under the Condition of the test.
- The heavy metals (Metallic impurities) may be iron, Copper, Lead, Nickel, Cobalt , bismuth and antimony etc.

❖ Principle:

- The Limit test for heavy metals has been based upon the reaction of the metal ion with hydrogen sulphide 0r Sodium sulphide under prescribed condition of the test causing the formation of metal sulphides.
- These remain distributed in a colloidal state and give rise to a brownish colouration.
- The colour produced in the test solution is compared with that of the standard solution containing a definite amount of lead.

$$\text{Heavy Metal (M)} + H_2S/N_2S \longrightarrow \text{Heavy Metal Sulphide (MS)} + H_2$$

- The metallic impurities in substances are expressed as parts per million of a substances. The Usual limit as per IP has been 20 ppm.

- The I.P has adopted 4 methods for limit tests for heavy metals.

❖ **Method I:**

- This Method is applicable for the sample which give clear colourless solution under specified condition of test.
- Method I based upon the reaction of the heavy metal ion with hydrogen sulphide leading to formation of heavy metal impurities.

TEST SOLUTION	STANDARD SOLUTION	REASON
Dissolve 4 gm of Sample (Dextrose) in 25 ml water and transfer to Nessler's Cylinder	Take 2 ml of Standard lead solution and dilute to 25 ml with water	Suspected sample is taken which might have an impurity of heavy metal and make its solution.
Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia Solution.	Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia Solution.	Maintain pH by acid or base.
Dilute with water to 35 ml.	Dilute with water to 35 ml.	Dilution for complete ionization

TEST SOLUTION	STANDARD SOLUTION	REASON
Add freshly prepared 10 ml H_2S Solution.	Add freshly prepared 10 ml H_2S Solution.	H_2S is added for precipitation.
Dilute with water to 50 ml.	Dilute with water to 50 ml.	Make up the same volume upto 5o ml in both for Comparison.
Allow to stand for 5 min. and view downwards on a white background.	Allow to stand for 5 min. and view downwards on a white background.	Compare the Intensity of colour in both.

- **Conclusion:** The Coloured Produced in the test solution should not greater than the Standard solution. If Colour Produced in the sample solution is less than the Standard solution the sample will pass the limit test of heavy metals.
- If Colour Produced in the sample solution is more than the Standard solution the sample will fail the limit test of heavy metals.

❖ Method II:

- This Method is applicable for the sample which do not give clear colourless solution under specified condition of test.
- Method II based upon the reaction of the heavy metal ion with hydrogen sulphide leading to formation of heavy metal impurities.

TEST SOLUTION	STANDARD SOLUTION
Weigh 2 gm Sample (Paracetamol) moisten with Sulphuric acid and ignite on a low flame till completely charred. (a) Add few drops of Nitric acid and heat to 500° C. Allow to cool and add 4 ml HCl and evaporate to dryness. (b) Moisten the residue with 10 ml of HCl and digest for 2 min. (c) Neutralize with Ammonia solution and make just acid with Acetic acid.	Take 2 ml of Standard lead solution and dilute to 25 ml with water

TEST SOLUTION	STANDARD SOLUTION
Adjust the pH 3 to 4 by adding dilute Acetic acid and dilute Ammonia Solution and filter if necessary	Adjust the pH 3 to 4 by adding dilute Acetic acid and dilute Ammonia Solution and filter if necessary
Dilute with Water 35 ml	Dilute with Water 35 ml
Add freshly Prepared 10 ml of hydrogen Sulphide Solution.	Add freshly Prepared 10 ml of hydrogen Sulphide Solution.
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for 5 Min.	Allow to stand for 5 Min.

- **Conclusion:** The Coloured Produced in the test solution should not greater than the Standard solution. If Colour Produced in the sample solution is less than the Standard solution the sample will pass the limit test of heavy metals.
- If Colour Produced in the sample solution is more than the Standard solution the sample will fail the limit test of heavy metals.

❖ Method III:

- This Method is applicable for the sample which give clear colourless solution in Presence of Sodium Hydroxide Solution.
- Method II based upon the reaction of the heavy metal ion with Sodium Hydroxide leading to formation of heavy metal impurities.

TEST SOLUTION	STANDARD SOLUTION
Solution is Prepared as per the Monograph and 25 ml is transferred in Nessler's Cylinder OR/ Weigh Specific amount of substance and dissolve in 20 ml of Water and add 5 ml of dilute NaOH Solution.	Take 2 ml of Standard Lead Solution.
Make up the Volume to 50 ml with Water	Make up the Volume to 50 ml with Water
Add 5 drops of Sodium Sulphide Solution.	Add 5 drops of Sodium Sulphide Solution.
Mix and set aside for 5 Min.	Mix and set aside for 5 Min.
View downwards over a white surface.	View downwards over a white surface.

❖ **Conclusion:** The Coloured Produced in the test solution should not greater than the Standard solution. If Colour Produced in the sample solution is less than the Standard solution the sample will pass the limit test of heavy metals.

➢ If Colour Produced in the sample solution is more than the Standard solution the sample will fail the limit test of heavy metals.

❖ **Method IV:** It is based upon Precipitation of relatively insoluble and characteristically coloured sulphides of heavy metals when Aqueous solutions are treated with alkali metal Sulphide.

❖ **Reagent Preparations:**

1) **Standard Solution:** Pipette 10 ml of Standard lead solution (2 ppm lead) into a Nessler's Cylinder. Add 2 ml of the test solution and mix.
2) **Test Solution:** Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder labelled as “Test”

3) **Preparation of Standard Lead Solution (2 ppm Pb):** Dilute 1 Volume of Standard lead solution (20 ppm Pb) to 10 Volume with Water.

4) **Preparation of Thioacetamide Reagent:** To 0.2 ml of 4% w/v Thioacetamide solution add 1 ml of a mixture of {15 ml of 1M NaOH, 5 ml of Water and 20 ml of Glycerine (85%)}. Heat on a water bath for 20 Sec. Cool and use immediately.

5) **Preparation of Acetate buffer of pH 3.5:** Dissolve 25 gm of Ammonium acetate in 25 ml of water and add 38 ml of 7M HCl. Adjust the pH to 3.5 by using 3 M HCl or 6 M Ammonia and dilute to 100 ml with water.

TEST SOLUTION	STANDARD SOLUTION	REASON
Prepare an Aqueous solution of sample as directed in the Monograph and Pipette out 12 ml transfer to Nessler Cylinder.	Take 10 ml of Standard lead solution in a Nessler Cylinder add 2 ml test solution and mix well.	Suspected sample is taken which might have an impurity of heavy metal and make its solution.
Add 2 ml of Acetate buffer of pH 3.5 and mix.	Add 2 ml of Acetate buffer of pH 3.5 and mix.	Maintain optimum pH for limit test.
Add 1.2 ml of Thioacetamide reagent and allow to stand for 2 Min. Brown coloured precipitates start to appear.	Add 1.2 ml of Thioacetamide reagent and allow to stand for 2 Min, Brown coloured precipitates start to appear.	Added for Precipitation of Metal Sulphides.
View downwards over a white surface.	View downwards over a white surface.	Compare Intensity of colour in both.

- **Conclusion:** The Coloured Produced in the test solution should not greater than the Standard solution. If Colour Produced in the sample solution is less than the Standard solution the sample will pass the limit test of heavy metals.
- If Colour Produced in the sample solution is more than the Standard solution the sample will fail the limit test of heavy metals.

❖ **Limit Test for Arsenic**: (Que: Write a S.N on Limit test for Arsenic or Gutzeit test) (Draw the Gutzeit Apparatus) (Write a S.N on Method use for Determine Arsenic Impurities

❖ **Principle:**

➢ The Limit test for Arsenic based on the Reduction of arsenic in arsenious state can be easily reduced Arsine gas (AsH_3). When this gas is passed over mercuric bromide paper it produce stain which ranges in colour form yellow to brown the intensity of stain and length of which are proportional to the amount of arsenic.

➢ B.P Suggest the use of a mercuric chloride paper instead of Mercuric bromide paper.

➢ The sample is dissolved in **acid** whereby the **arsenic present as impurity** in the sample gets converted to **arsenic acid.** The **arsenic acid is reduced to arsenious acid** by **reducing agents** like stannous acid**, Potassium iodide** etc.

➢ The **nascent** hydrogen (Monatomic Hydrogen) formed during the reaction is reduced from **arsenious acid to the arsine gas.**

- The arsine gas react with mercuric chloride paper to produce yellow –brown stain.

$$AS^{3+} \xrightarrow{HCl} H_3AsO_4$$

(Impurity) (Arsenic acid)

$$H_3AsO_4 \xrightarrow{KI} H_3AsO_3 + 3H_2 \xrightarrow{\text{Nascent hydrogen}} AsH_3 + 3H_2O$$

(Arsenic acid) (Arsenious acid) (Arsine gas)

$$AsH_3 + HgCl_2 \longrightarrow Hg(AsH_2)_2 + 2HCl$$

(Arsine) (Mercuric Chloride) (Yellow stain)

- The depth of the yellow stain depending upon the amount of arsenic present in the sample is compared with that of standard stain produced from a known amount of arsenic.

Reagent Preparation:

1) Preparation of test Solution: The solution of water soluble substance is prepared with water and standard HCl AsT (Suitable for Arsenic test). The solution of substance such as metallic carbonates, which effervesce with acid is obtained with Standard HCl AsT. The Substances which are insoluble e.g $BaSO_4$, Bentonite are diffused in water.

2) Stannous Chloride solution AsT: It is Prepared by adding Stannous chloride solution to an equal volume of HCl AsT reducing to the original volume by boiling and filtering through a fine-grain filter paper.

3) Stannated HCl AsT: It is Prepared by adding 1 ml of Stannous chloride solution AsT to 100 ml of HCl acid AsT.

4) Preparation of Standard arsenic solution (10 ppm As): Dissolve 0.330 gm of Arsenic Trioxide (ArO_3) in 5 ml of 2M NaOH and dilute to 150 ml water. Dilute 1 Volume of this solution to 100 ml with water.

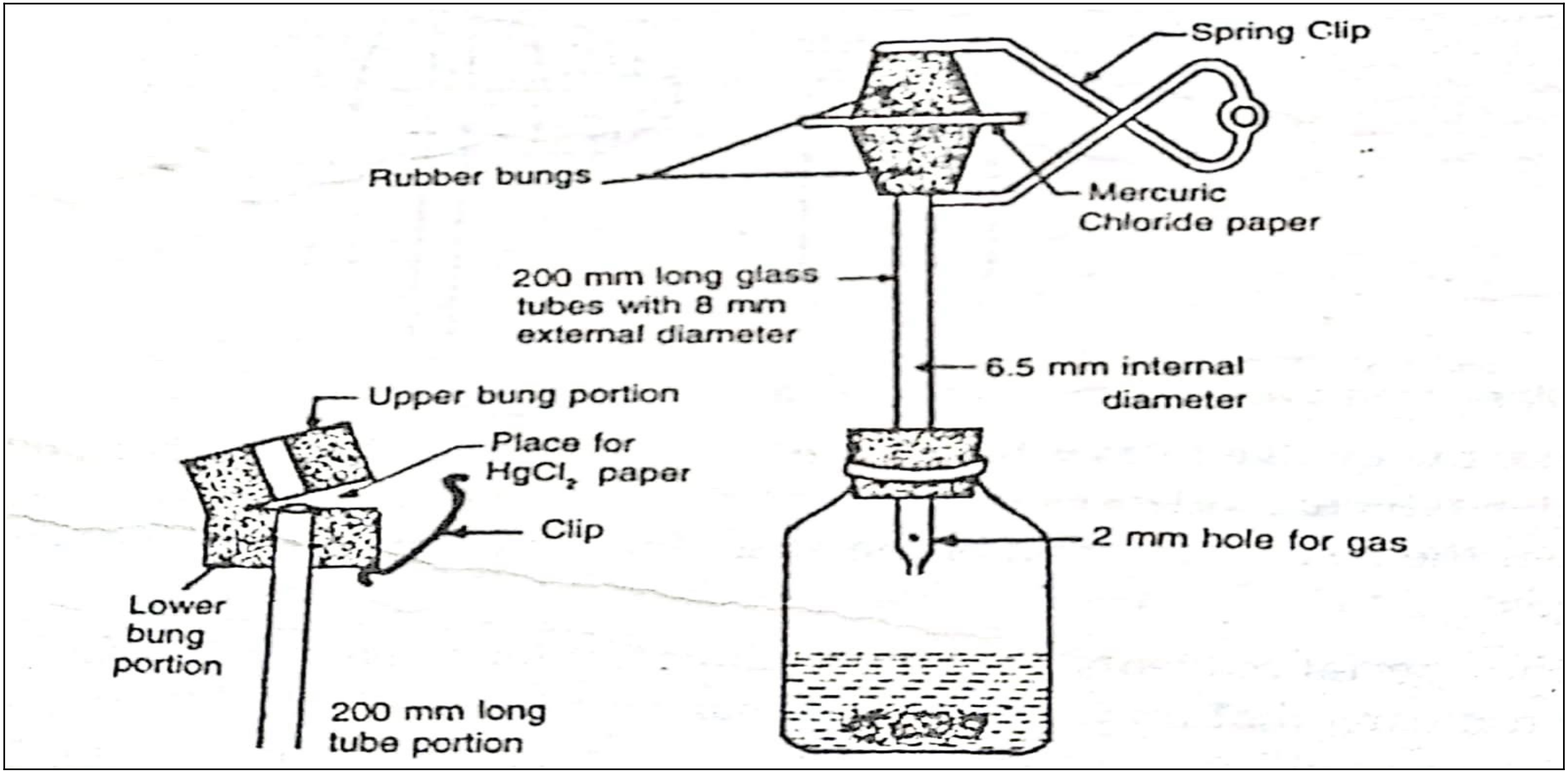
5) Zinc AsT: It is the granulated zinc which complies with the following additional test: To 10 gm of the granulated zinc add 15 ml of Stannous chloride solution AsT and 5 ml 0.1 M Potassium iodide. Apply the general test but allow the reaction to continue for 1 hour. No visible stain should be produced on mercuric chloride paper. Repeat the test by adding 0.1 ml of standard arsenic solution (10 ppm) a faint but distinct yellow stain is produced.

❖ Method for the Limit test for Arsenic:

❖ Procedure:

TEST SOLUTION	STANDARD SOLUTION	REASON
Dissolve 2.5 gm of sample in 50 ml water and place it in bottle of an arsenic test apparatus labelled as 'Test'	1 ml of arsenic Standard Solution (10 ppm As) is diluted to 50 ml Distilled water.	Suspected sample is taken which might have an impurity of arsenic and make its solution as per monograph.
5 ml of 1M Potassium iodide + 5 ml of Stannated HCl solution AsT + 10 gm of Zinc AsT are added.	5 ml of 1M Potassium iodide + 5 ml of Stannated HCl solution AsT + 10 gm of Zinc AsT are added.	(a) Stannous chloride is used for complete evolution of arsine gas. (b) Zinc, Potassium iodide and stannous chloride are used as a reducing agent. (c) HCl is used to make the solution acidic.
Immediately the apparatus is assembled and the flask is immersed in water bath at a Tem. Of 40° C.	Immediately the apparatus is assembled and the flask is immersed in water bath at a Tem. Of 40° C.	Uniform evolution of arsine gas maintained at 40° C.
After 40 Min. any Stain Produced on the Mercuric chloride paper is observed.	After 40 Min. any Stain Produced on the Mercuric chloride paper is observed.	Arsine gas react with $HgCl_2$ and make a yellow stain. Compare the intensity of yellow colour in both.

- **Note:** The Stain Produced on Paper fades on Keeping and therefore the stain should be compared Immediately. Arsine gas Produced is passed through lead acetate solution to purify and trap other impurities like H_2S gas.
- **Conclusion:** After 40 Min. If the Intensity of yellow stain produced in standard is more than that in the test the sample Complies (Pass) limit test of Arsenic.
- After 40 Min. If the Intensity of yellow stain produced in standard is less than that in the test the sample does not Complies (Fail) limit test of Arsenic.



ARSENIC APPARATUS

❖ Construction:

1. It is having a wide mouthed glass bottle of 120 ml capacity having mouth about 2.5 cm in diameter.
2. This bottle is fitted with a rubber bung through which passes a glass tube, 20 cm long having external diameter about 0.8 cm and Internal diameter of 0.65 cm.
3. The tube is constricted at its lower end extremity to about 1 mm diameter and there is blown a hole not less than 2 mm in diameter in the side of the tube near the constricted part.
4. The upper end of the glass tube has been fitted with two rubber bungs (about 25 mm × 25 mm) each having a hole bored centrally and exactly 6.5 mm in diameter.
5. One of the bungs has been fitted to the upper end of the tube while the second bung has to be fitted upon the first bung in such a way that the mercuric chloride paper gets exactly sandwiched between the central perforation of the two.
6. The bungs are the kept in close contact by using rubber band or spring clip in such a manner that gas evolved from bottle must have to pass through the 0.65 mm internal circle of mercuric chloride paper.

7. During the test, the evolved gases have been passing through the side hole, the lower hole serving as an exit for water which condenses in the constricted Part of the Tube.
8. An Important feature of the apparatus has been the standardisation of the area of mercuric chloride paper which is exposed to the action of arsine gas.

❖ QUESTIONS:

1. **Define Impurities**. Enlist the sources of the impurities in Pharmaceuticals and discuss the manufacturing hazards as source of impurity.
2. What do you understand by limit test? Give its importance in pharmacy. **Explain principle and procedure for limit test for Iron.**
3. **Draw a neat and labeled diagram of Gutzeit apparatus.**
4. **Discuss the source of Impurities.**
5. Write down principle of Limit test of chloride and sulphate.
6. **Write short note on pharmacopoeia**? Brief on Indian Pharmacopoeia.
7. Define limit test. **Write a detailed note on limit test of Iron.**
8. **Write limit test for arsenic.**
9. What is Pharmacopoeia? Write a brief note on monograph.


THANK
YOU