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Impurities in Pharmaceutical Substances

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Introduction

- Inorganic chemistry : It is study of all elements and their compounds except carbon and its compounds obtained from non living things and minerals found in earth.

- Pharmaceutical inorganic chemistry : It deals with study of essential and non essential elements their preparation, standards of purity, test for identification limit test to be performed for determining quality, extent of purity, different formulations, their storage conditions and therapeutic uses.

PHARMACOPOEIA

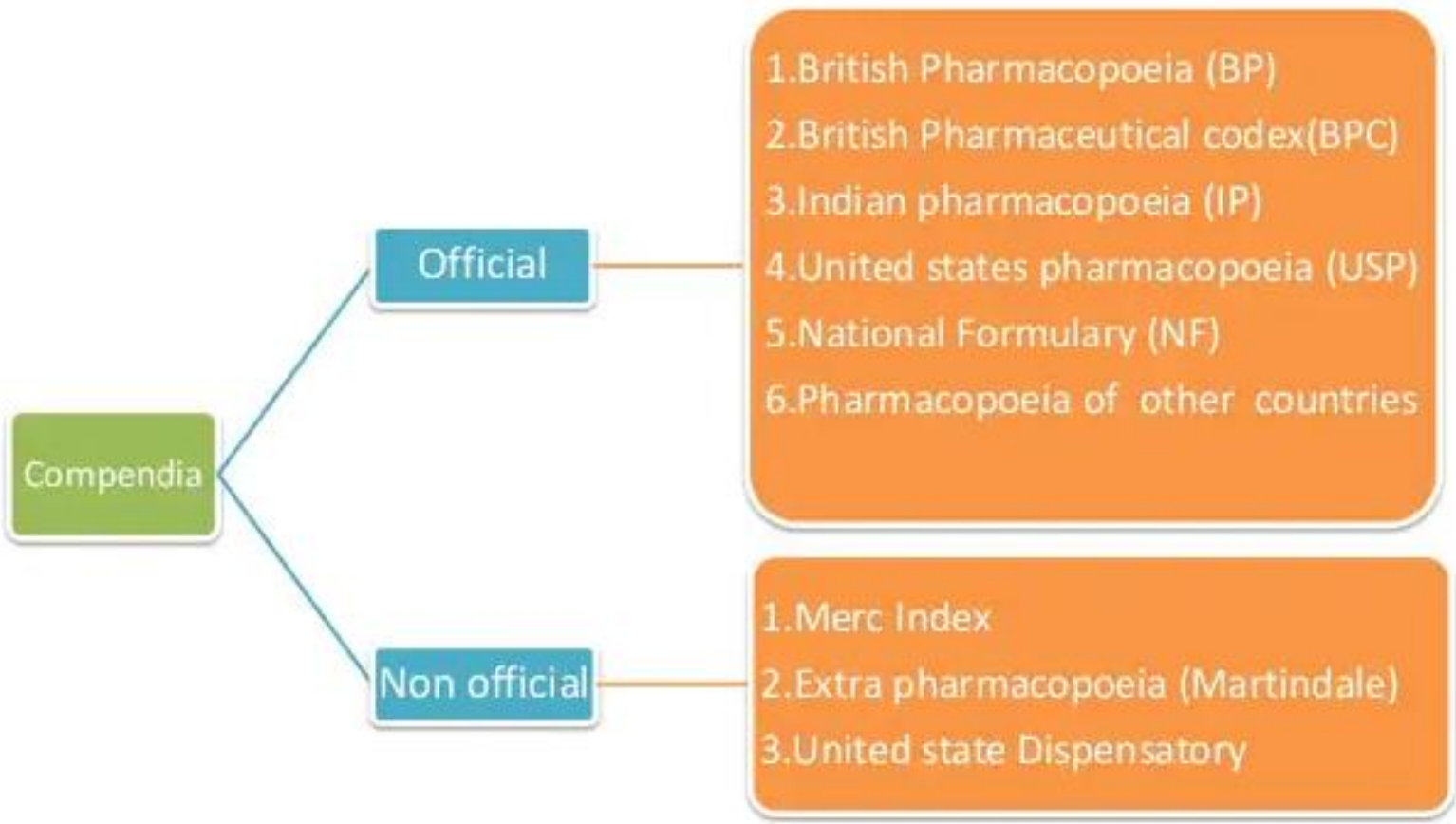
Pharmcon-Drug or medicine

Poeia- To make

Prepared by; Government authority of respective countries

The book containing the standards of drug and other related substances are known as pharmacopoeia and formularies Collective name- Drug compendia Book revised from time to time	**Contains**: 1.List of drugs 2.Related substances 3.Sources,description,standard Test, formulae for preparing same, mode of action, uses, doses, storage condition etc.	**Preparation of book**: Experts in the field like Medical practitioners, Teachers, scientist, pharmaceutical manufacturers

Classification of compendia



History of Pharmacopoeia

The term Pharmacopoeia first distinct title in work published in Basel, Switzerland in 1561 by Dr. A.Foes

On 15th December 1820,First United state pharmacopoeia was released (U.S.P)

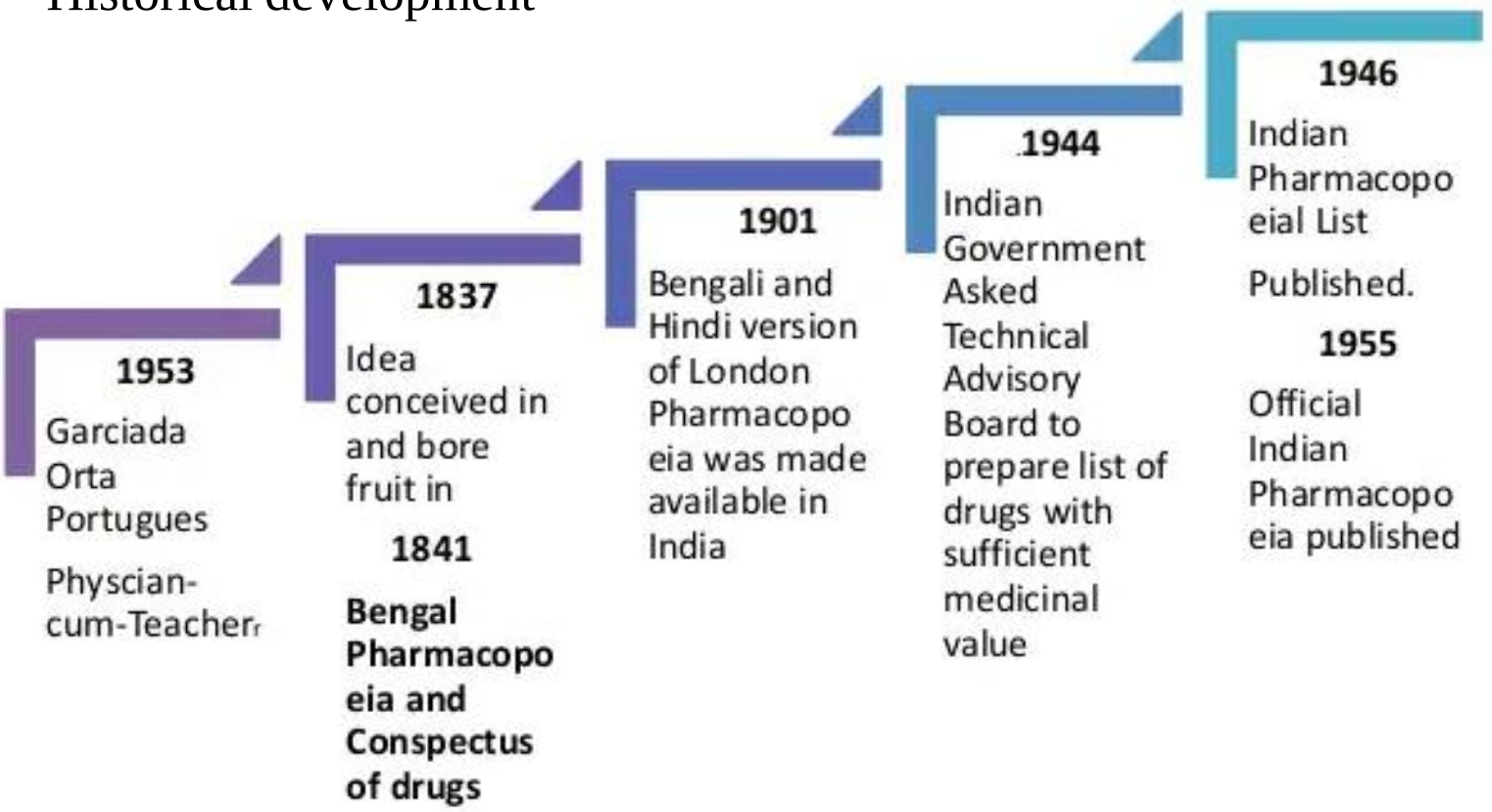
In 1864 the first British Pharmacopoeia(B.P) was published inclusion on few monographs

Today's pharmacopoeias focus mainly on assurance of quality of products by various tools of analytical sciences.

INDIAN PHARMACOPOEIA

INDIAN PHARMACOPOEIA

Historical development



Indian Pharmacopoeia

Indian Pharmacopoeial List 1946:

- List of drugs included and not included in British Pharmacopoeia along with standards published by Government of India under name of " The Indian Pharmacopoeial list"
- Committee constituted under chairmanship of Col. Sir. R. N. Chopra along with Nine members

List Consist of Following details

a) Drugs of plant and animal origin
b) Biological products
c) Insecticide
d) Coloring agents
e) Synthesis
f) Miscellaneous
g) Drugs of veterinary use

Pharmacopoeial description/presentation

A) Introduction including general notices

B) Monographs of official drugs

C) Appendices and index

A) Introduction including general notices

- Summarize different changes including addition/deletion
- Comparison bet current and last edition
- Avoids misinterpretation and misunderstanding the text attention should be paid to general notices.

b) Monographs:

- Written study of subject implied by word “monograph”
- General monographs of dosage form and API grouped together at beginning of Vol. II of IP 2010 in alphabetical order.
- Monographs related to herbal products, blood and blood related products, biotechnological products, veterinary products given in separate sections Volume III of IP 2010.

Monographs Contains:

1. Main Title: Main Name of substances
2. Synonym: Common name of substance
3. Chemical Formula and Mol.Wt: As per IUPAC
4. Category: Uses of drug e.g Antibacterial,
5. Doses: Average range of quantity for adults
6. Description: General physical properties

Monographs Contains:

7. Solubility: Approximate part of solvent for 1 part of solute
8. Standards: Standards for purity and strength e.g NaHCO3 NLT 99 and NMT 100.5 %
9. Identification: Specific and Non specific test for identity
10. Test for Purity: m.p, b.p, wt per ml, limit test, sulpahted ash
11. Assay Method: Quantitative determination of principle ingredient
12. Storage: Conditions for storage to prevent deterioration

PHARMACOPOEIAL DESCRIPTION/PRESENTATION

C) Appendices and Index: Includes general notices and monographs apparatus needed for the tests and assay it includes

Appendices and Index

- ✓ Infra-red spectra
- ✓ Apparatus for test and assay
- ✓ Biological tests and determinations
- ✓ Chemical test and assay
- ✓ Chromatography and electrophoresis
- ✓ Clarity and color solutions
- ✓ Dissolution and disintegration
- ✓ Microbiological assay
- ✓ Limit Test of particulate matter

Impurity

- **Impure Chemical Compound:** A compound is said to be impure if it is having foreign matter i.e. Impurities.
- **Pure Chemical Compound:** A pure chemical compound refers to that compound which is having no foreign matter i.e. impurities.
- Chemical purity means freedom from foreign matter.
- Analytically 100 % pure substances are not available and traces of impurities must be present.
- Normally undesirable foreign materials are present in the pharmaceutical substances.

What is impurity?

- Any material that affects the purity of the material of interest.

Impurity means undesired particles

- Presence of Impurities in the pharmaceutical substances may produce toxic effects on the body and may also lower down the active strength of the pharmaceutical substance.
- Impurities commonly in chemical substances include small quantities of lead, Arsenic, Iron, Chloride and sulphate.

Impurity

- Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions.
- Chemical purity means freedom from all foreign materials. Purification of chemicals is expensive and therefore purifying a substance to much higher degree is necessary.

Effects of Impurities

Pure substances are difficult to get and some amount of impurity is always present in the material. So the impurities which are present in the substances may have the following effects:

1. Impurities may lower the shelf life of the substances.
2. Therapeutic effect can be decreased.
3. Impurities may bring about incompatibility with other substances.
4. Impurities may cause difficulties during formulations and use of the substances.
5. Sometimes Impurities changes the chemical and physical properties of the substances.
6. Shows toxic effect after a certain period

Sources of Impurity:

- A compound having foreign materials is said to be impure.
- The origin of impurities in drugs is from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms. Majority of the impurities are characteristics of the synthetic route of the manufacturing process.
- The pharmaceutical preparation should be free from toxic and other impurities.
- The impurities commonly found in medicinal preparations are:
- Impurities due to which substances become incompatible. Due to colouring or flavouring substances, e.g., Sodium Salicylate.
- Humidity.
- Chemical and physical properties.

The Various Sources Of Impurities In Pharmaceutical Substances Are As Follows:

1. **Raw Materials Used in the Manufacturing of Pharmaceutical Process:**

   The source of pharmaceutical substances are either natural or synthesized from chemical starting materials. It is essential to verify the identity of the source material and its quality otherwise it contaminate the final

   Example:

- ✓ lead and heavy metals are found as impurities in many sulphide ores,
- ✓ Rock salt used for the preparation of sodium chloride is contaminated with small amounts of calcium and magnesium chlorides.

2. Reagents employed in the manufacturing process:

Pharmaceutical substances are either isolated from natural sources (mineral sources, plants, animals and microbes) or synthesized from chemical starting materials. If reagents are employed in the manufacturing process are not completely removed by washing, these reagents may be present in final products.

Example: e.g., Magnesium impurities are found in calcium minerals, aluminum ores
are usually accompanied by alkali and alkaline earth compounds.

3. **Solvents:** Water is the most commonly used solvents in the preparation of inorganic pharmaceuticals. Different types of water containing different types of impurities. Various types of water are:

a. **Tap water:** Containing impurities of Magnesium, sodium, calcium, chloride, sulphates and carbonates.
b. **Softened water.** It is prepared from tap water nut it contains more of sodium and chloride ions as impurities.
c. **Demineralized water.** It is prepared by ion exchange and it is free from Magnesium, sodium, calcium, chloride, sulphates and carbonates impurities. It contains pyrogens, bacteria and organic impurities.
d. **Distilled water:** This water is free from all inorganic and organic impurities. It is best solvent for pharmaceutical preparations.

4. **Action of reagents on reaction vessels:**

   Reaction vessels used in the manufacturing process may be metallic such as iron, cast iron, galvanized iron, copper, silver, aluminium, nickel, zinc and lead.

5. **Atmospheric contamination during manufacturing process:** Atmosphere may contain dust (sulphur, aluminum oxide, silica, soot etc.) and some gases like carbon dioxide, sulphur dioxide, arsine and hydrogen sulphide. These may contaminate the final product during the manufacturing process.

6. Chemical process used in the manufacture:

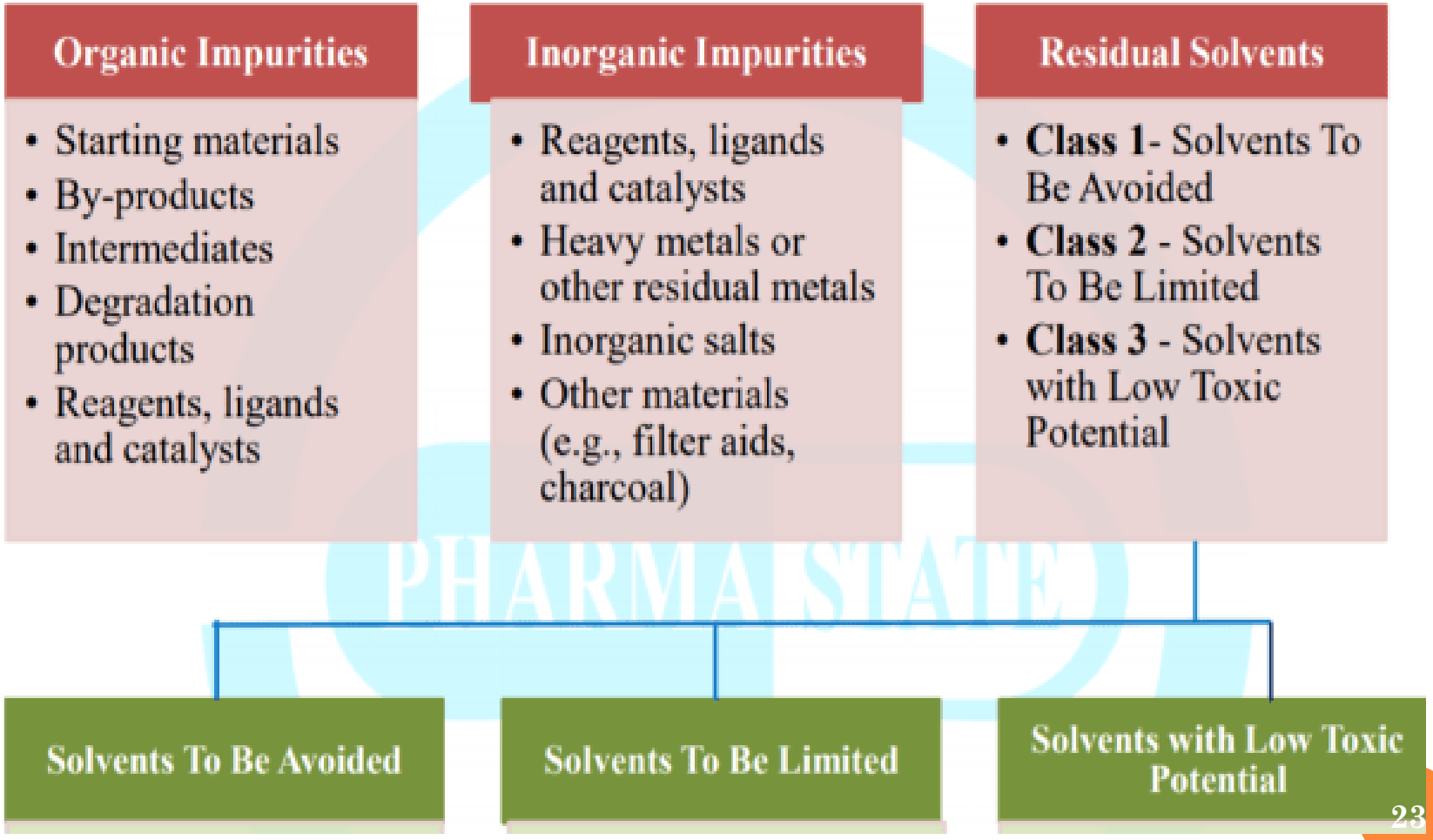
Various chemical reactions such as oxidation, nitration, reduction, halogenations, hydrolysis are involved for the synthesis of drugs. In these chemical reactions various chemicals and tap water is used it is often having Mg+2, Ca+2 and Cl, which are generally found in the substance which is being manufactured.

7. Defects in the manufacturing process:

Defects like incompleteness, pH, pressure, temperature and imperfect mixing in various manufacturing processes produce impurities in chemical compounds.

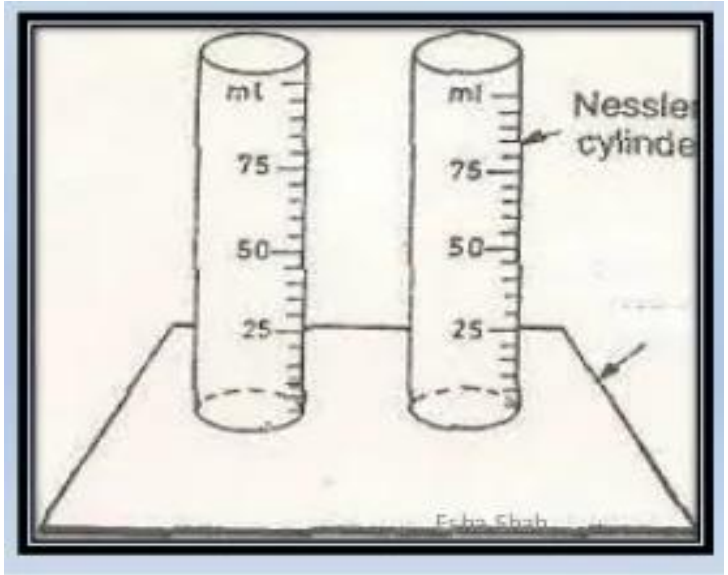
To prevent these impurities many test such as limit test are carried out to lower the impurities and to make the pharmaceuticals more safe.

Types of Impurities



LIMIT TESTS:

- Tests being used to identify the impurity.
- Tests being used to control the impurity.
- **Definition:** Limit tests are quantitative or semi quantitative test designed to identify and control small quantities of impurities which are likely to be present in the substances.



Factors affecting limit tests:

- Specificity of the tests
- Sensitivity
- Control of personal errors (Analyst errors)
- Test in which there is no visible reaction
- Comparison methods
- Quantitative determination

Turbidity means cloudiness or haziness.

LIMIT TEST FOR CHLORIDE:

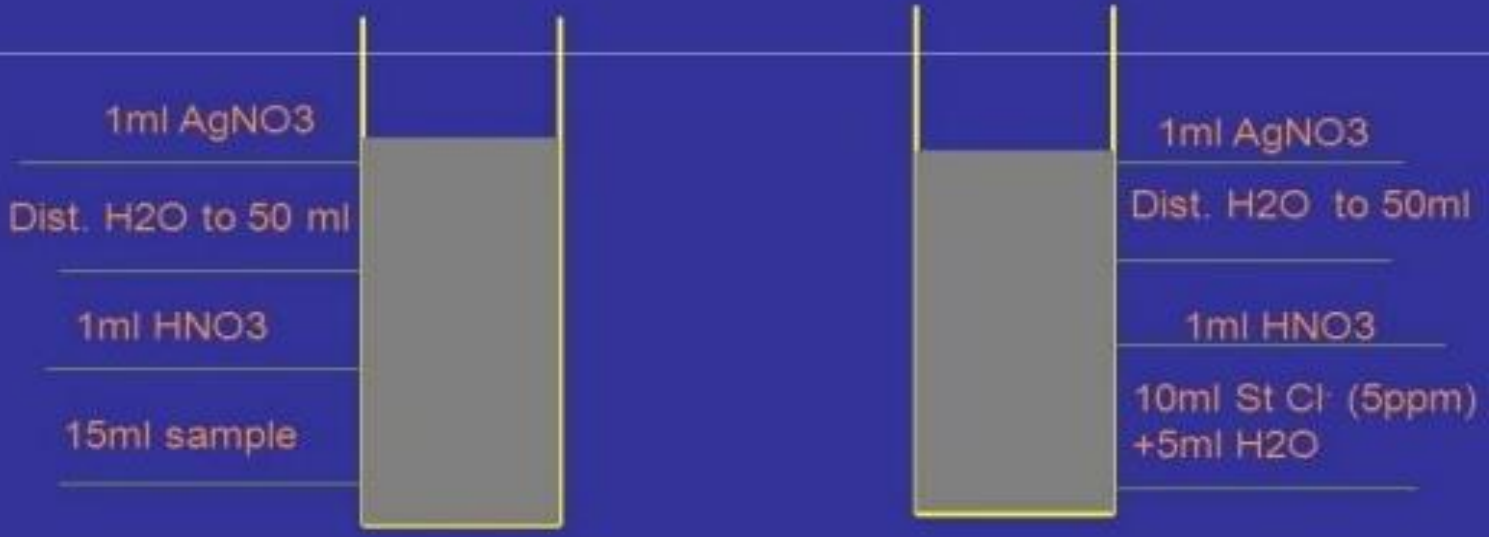
- Principle:

  Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in presence of dilute nitric acid to form silver chloride, which appears as solid particles (Opalescence) in the solution.

Limit Test for Chloride

Principle:

$Cl^- + AgNO_3 \longrightarrow AgCl$



Test sample	Standard compound
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder	Take 1 ml of 0.05845 % W/V solution of sodium chloride in Nessler cylinder
Add 1 ml of nitric acid	Add 1 ml of nitric acid
Dilute to 50 ml in Nessler cylinder	Dilute to 50 ml in Nessler cylinder
Add 1 ml of $AgNO_3$ solution	Add 1 ml of $AgNO_3$ solution
Keep aside for 5 min	Keep aside for 5 min
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

- **Observation:** The opalescence produce in sample solution should not be greater than standard solution. If opalescence produces in sample solution is less than the standard solution, the sample will pass the limit test of chloride and visa versa.

- **Reasons:** Nitric acid is added in the limit test of chloride to make solution acidic and helps silver chloride precipitate to make solution turbid at the end of process as Dilute HNO_3 is insoluble in AgCl.

Modified chloride limit test

Glassware's required:

1) Nessler cylinder
2) Measuring cylinder
3) Glass rod

Chemicals required:

1) Dilute nitric acid
2) 0.1M Silver chloride
3) Conc. HCl
4) Distilled water

Principle: limit test of chloride is based on the precipitation reaction. The precipitates of chlorides develop on reaction of soluble chloride with silver nitrate in the presence of dilute nitric acid to form silver chloride, which appears as solid particles (opalescence) in the solution. The intensity of turbidity depends on the amount of chlorides present in test substance.

Procedure: 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 sodium chloride(NaCl, known Cl- impurity) but now it has been modified by using hydrochloric acid (HCl) instead of sodium chloride(NaCl).

$$HCl + AgNO3 \rightarrow AgCl + HNO3$$

Conclusion : If opalescence produced in sample solution is less than the standard solution, the sample will pass the limit test of chloride.

LIMIT TEST FOR SULPHATE:

- The Sulfate Limit Test is designed to determine the allowable limit of sulfate contained in a sample.
- **Principle:** Limit test of sulphate is based on the reaction of soluble sulphate with barium chloride in presence of dilute hydrochloric acid to form barium sulphate which appears as solid particles (turbidity) in the solution.

$$SO4 + BaCl2 \text{ ----------} \rightarrow BaSO4 + 2Cl\text{-}2 \text{ -}$$

PROCEDURE

Test sample	Standard compound
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder	Take 1 ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder
Add 2 ml of dilute hydrochloric acid	Add 2 ml of dilute hydrochloric acid
Dilute to 45 ml in Nessler cylinder	Dilute to 45 ml in Nessler cylinder
Add 5 ml of barium sulphate reagent	Add 5 ml of barium sulphate reagent
Keep aside for 5 min	Keep aside for 5 min
Observe the Turbidity	Observe the Turbidity

- **Observation:** The turbidity produce in sample solution should not be greater than standard solution. If turbidity produces in sample solution is less than the standard solution, the sample will pass the limit test of sulphate and vice versa.

- **Reasons:** Hydrochloric acid helps to make solution acidic. Potassium sulphate is used to increase the sensitivity of the test by giving ionic concentration in the reagent. Alcohol helps to prevent super saturation and so produces a more uniform opalescence.

Modified limit test for sulphates

Glassware required:

1) Nessler cylinder
2) Measuring cylinder
3) Glass rod

Chemicals required:

1) Potassium sulphate
2) Test substance
3) Hydrochloric acid
4) Barium sulphate reagent
5) Distilled water

Principle: The principle involved in the limit test for sulphate is precipitation method. The sulphates are precipitated as barium sulphate by reacting with barium chloride in the presence of hydrochloric acid. The HCl used prevents the reaction of other acid radicals with barium chloride as in the presence of hydrochloric acid, only sulphates are precipitated.

$$SO_4^{2-} + BaCl_2 \rightarrow BaSO_4 + 2Cl^-$$

Due to the formation of precipitates, the solution appears turbid and the extent of turbidity depends on the amount of sulphates present. If the turbidity produced by the test is less than that of standard, it means that the sample contains sulphates within prescribed limits.

Procedure: From IP.1996 onwards limit test for sulphate has been modified to 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.

Reagent preparation:

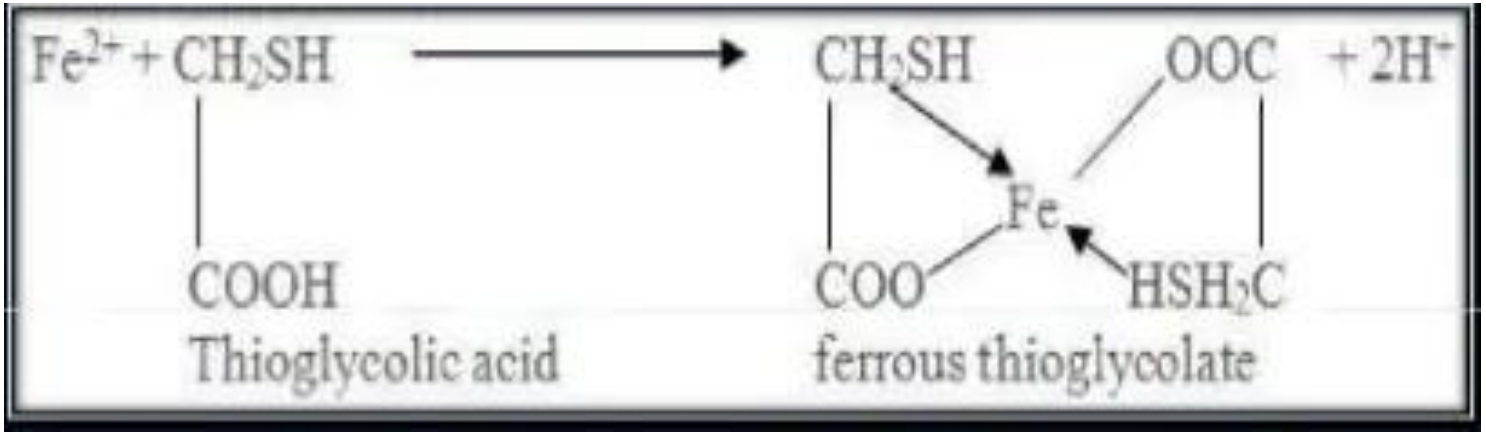
- Barium sulphate reagent: Dissolve 12 g of $BaCl_2 .2H_2O$ in 1000 ml of water to make 0.05 M barium chloride solution. To 15 ml of the prepared solution, add 55 ml water, 20 ml alcohol, 5 ml of 0.0181% w/v K_2SO_4 solution and makeup the volume upto 100 ml.
- Standard potassium sulphate solution: Accurately weigh 0.1089 g of K_2SO_4 and the volume was makeup up to 100 ml with water.

- Test sample: Sodium chloride: Dissolve 2 g of sodium chloride in 20 ml of water.
- Sodium bicarbonate: Dissolve 2 g of sodium bicarbonate in little quantity of water.

Conclusion: If opalescence produced in the standard is more than that of test solution, the sample complies the limit test of sulphate as per IP. 1996.

LIMIT TEST FOR IRON:

- Limit test of Iron is based on the reaction of iron in ammonical solution with thioglycollic acid in presence of citric acid to form iron thioglycolate (Ferrous thioglycolate complex) which produces pale pink to deep reddish purple color in alkaline media.
- Thioglycolic acid is used as reducing agent.



PROCEDURE

Test sample	Standard compound
Sample is dissolved in specific amount of water and then volume is made up to 40 ml	2 ml of standard solution of iron diluted with water upto 40 ml
Add 2 ml of 20 % w/v of citric acid (iron free)	Add 2 ml of 20 % w/v of citric acid (iron free)
Add 2 drops of thioglycollic acid	Add 2 drops of thioglycollic acid
Add ammonia to make the solution alkaline and adjust the volume to 50 ml	Add ammonia to make the solution alkaline and adjust the volume to 50 ml
Keep aside for 5 min	Keep aside for 5 min
Color developed is viewed vertically and compared with standard solution	Color developed is viewed vertically and compared with standard solution

- **Observation:** The purple color produce in sample solution should not be greater than standard solution. If purple color produces in sample solution is less than the standard solution, the sample will pass the limit test of iron and vice versa.

- **Reasons:**

- Citric acid forms complex with metal cation and helps precipitation of iron by ammonia by forming a complex with it.
- Thioglycolic acid helps to oxidize iron (II) to iron (III).
- Ammonia is added to make solution alkaline. The pale pink color is visible only in the alkaline media. The color is not visible in acidic media as ferrous thioglycolate complex decomposes in high acidic media.

Limit test for Heavy Metals :

- **Principle:** Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulphide in acidic medium to form **brownish colour solution** response to this test are lead, mercury , bismuth, arsenic, antimony , tin, cadmium, silver , copper , and molybdenum.
- The metallic impurities in substances are expressed as parts of lead per million parts of the substance. **The usual limit as per Indian Pharmacopoeia is 20 ppm**

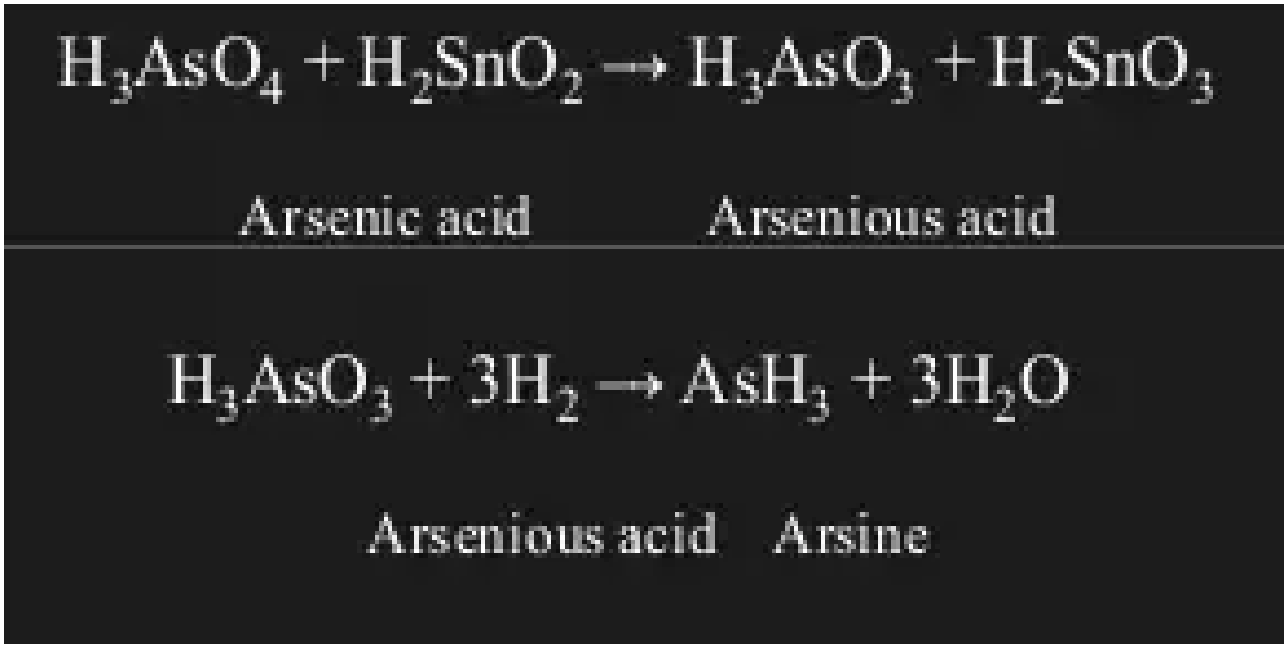
- Procedure: The I.P. has adopted three methods for the limit test of heavy metals.

Test sample	Standard compound
1. Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder	1. Take 2 ml of standard lead solution and dilute to 25 ml with water
2. Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'	2. Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'
3. Dilute with water to 35 ml	3. Dilute with water to 35 ml
4. Add freshly prepared 10 ml of hydrogen sulphide solution	4. Add freshly prepared 10 ml of hydrogen sulphide solution
5. Dilute with water to 50 ml	5. Dilute with water to 50 ml
6. Allow to stand for five minutes	6. Allow to stand for five minutes
7. View downwards over a white surface	7. View downwards over a white surface

- **Observation:** The color produce in sample(Test) solution should solution. If color produces in sample solution is sample will pass the limit test of heavy metals & vice versa.

Limit Test of Arsenic

- **Principle:** Limit test of Arsenic is based on the reaction of **yellow stain on mercuric chloride paper** in presence of potassium iodide. It is also called as Gutzeit test. Arsenic present as arsenic acid in the sample is reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid Arsenious acid is further reduced to arsine (gas) mercuric chloride paper to give a **yellow stain**



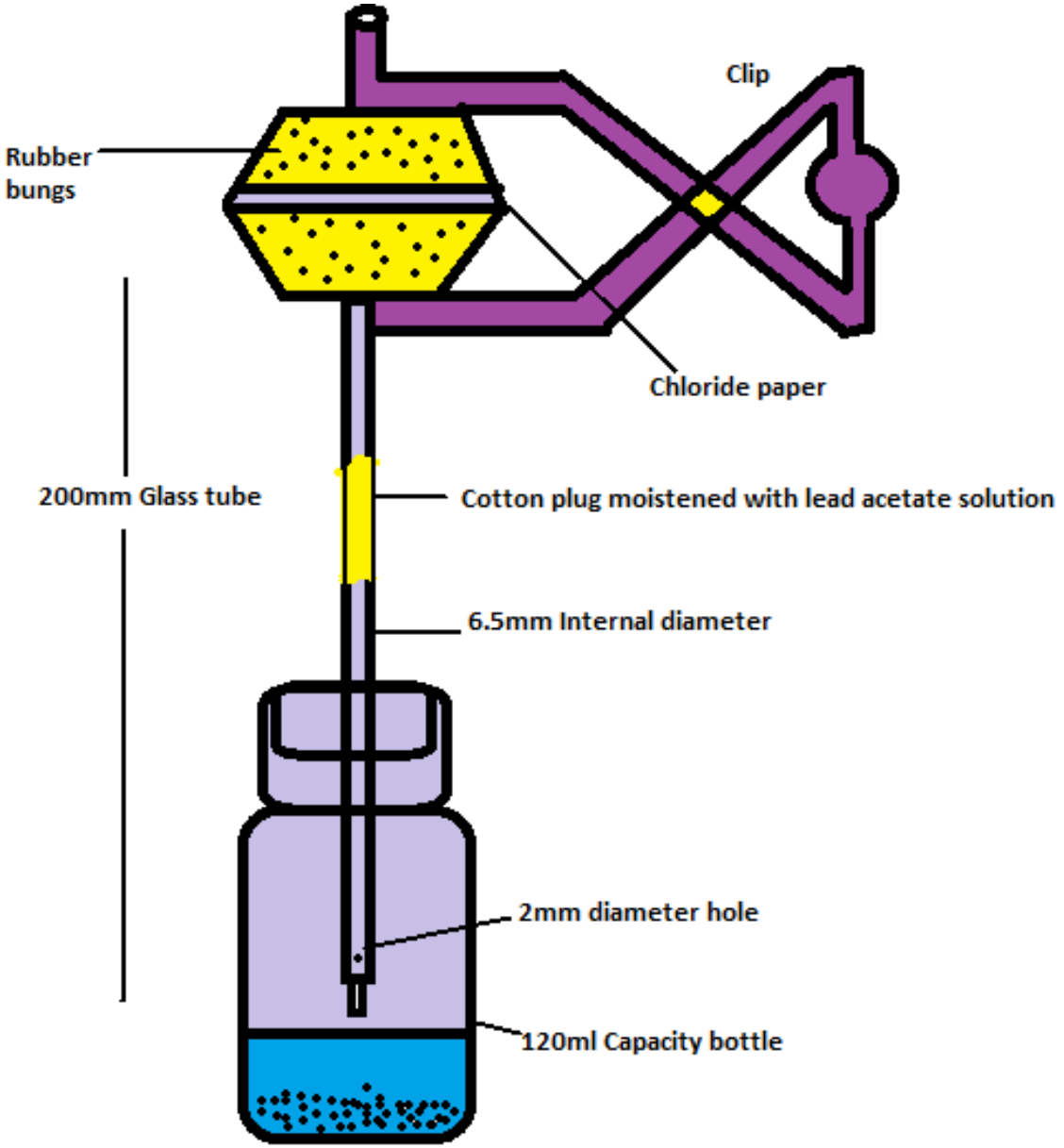
The **depth of yellow stain** on mercuric chloride paper will depend upon the quality of the arsenic present in the sample

Procedure:

Test solution: The test solution is prepared by dissolving specific amount in water and stannated HCl (arsenic free) and kept in a wide mouthed bottle. To this solution 1 gm of KI, 5 ml of stannous chloride acid solution and 10 gm of zinc is added (all this reagents must be arsenic free) Keep the solution aside for 40 min and stain obtained on mercuric chloride paper is compared with standard solution.

Standard solution: A known quantity of dilute arsenic solution is kept in wide mouthed bottle and rest procedure is followed as described in test solution.

APPARATUS USED FOR ARSENIC LIMIT TEST



Limit test for Lead:

- Lead is a most undesirable impurity in medical compounds and comes through use of sulphuric acid, lead lined apparatus and glass bottles use for storage of chemicals.
- **Principle:** Limit test of lead is based on the reaction of lead and diphenylthiocabazone (Dithizone) in alkaline solution to form lead Dithizone complex which is red in color. Dithizone is green in color in chloroform and lead- Dithizone complex is violet in color, so the resulting color at the end of process is red.

Standard Preparation	Test Preparation
1. Take specified volume of standard lead solution in a separating funnel as prescribed in the pharmacopoeia.	1. Take the specified volume of a sample solution in a separating funnel.
2. Add 6ml of ammonium citrate solution.	2. Add 6ml of ammonium citrate solution.
3. Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride solution.	3. Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride solution.
4. Add 2 drops of phenol red indicator.	4. Add 2 drops of phenol red indicator.
5. Make the solution alkaline by adding ammonia solution.	5. Make the solution alkaline by adding ammonia solution.
6. Extract with 5 ml of dithizone until it becomes green.	6. Extract with 5 ml of dithizone until it becomes green.
7. Combine dithizone extracts are shaken for 30mins with 30 ml of nitric acid and the chloroform layer is discarded.	7. Combine dithizone extracts are shaken for 30mins with 30 ml of nitric acid and the chloroform layer is discarded.
8. To the acid solution add 5 ml of standard dithizone solution.	8. To the acid solution add 5 ml of standard dithizone solution.
9. Add 4 ml of ammonium cyanide.	9. Add 4 ml of ammonium cyanide.
10. Shake for 30 min.	10. Shake for 30 min.
11. Compare the color(red) developed.	11. Compare the color(red) developed.

- **Observation:** The intensity of the color of complex, is depends on the amount of lead in the solution. The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of lead and vice versa.

Reasons:

- Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated.
- Phenol red is used as indicator to develop the color at the end of process.
- Lead present as an impurities in the substance, gets separated bye extracting an alkaline solution with a dithizone extraction solution.

Thank you