# Impurities in Pharmaceuticals and Limit test

# Outline of the chapter

**Definition of impurity**

**Sources of Impurity**

**Definition of Assay**

**Impurities commonly found in medicinal preparations**

**Definition of limit test**

**Importance of Limit test in pharmaceuticals**

**Limit test for Iron, Arsenic, Chloride, Lead, Sulphate, Heavy metals**

## 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.

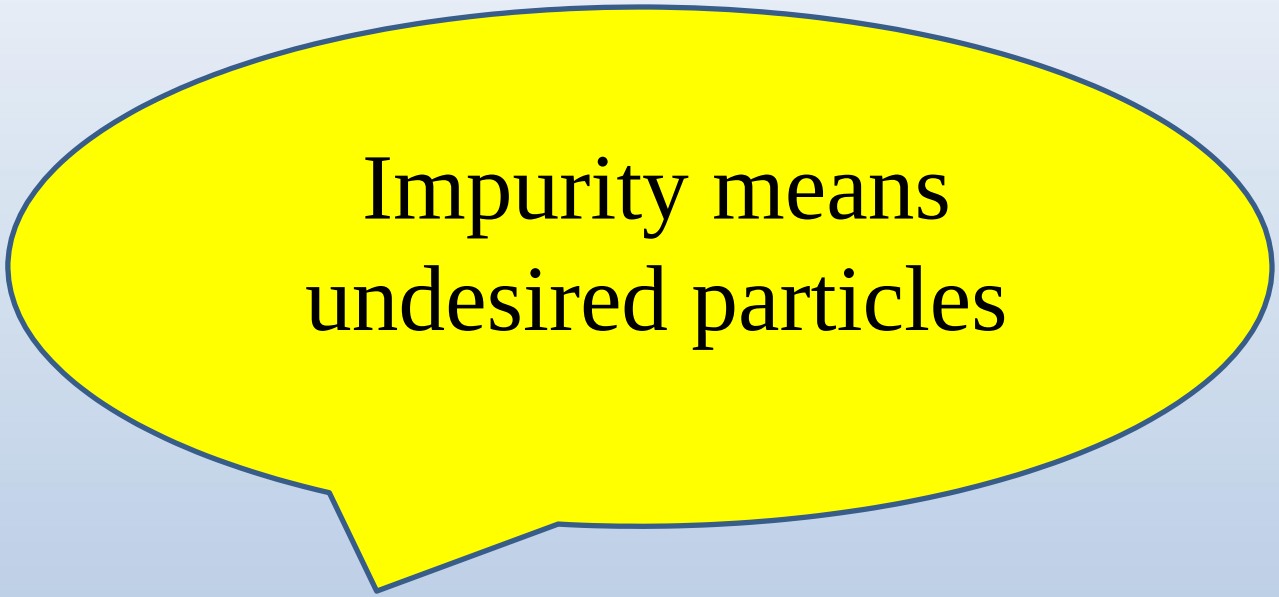
Chemical purity means freedom from foreign matter.

***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.




- ***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.***

# Impurities commonly found in medicinal preparations:

- Impurities which have **toxic effects** on body and bring about unpleasant reactions when present beyond certain limits. e.g Lead and Arsenic salts.
- The impurities which are able to make substance incompatible with other substances.
- The impurities which if present beyond the limit, affect the storage property of the pharmaceuticals.
- The impurities which are harmless, but if present beyond the limit, it will lower the active strength of the medicinal compound. E.g Sodium salt in potassium salt.
- The impurities which may bring about technical difficulties in the use of the substance.
- Impurities such as taste, odour, colour or appearance which can be easily detected by the senses and make the substance unhygienic and unaesthetic. E.g. Sodium chloride becomes damp because of the presence of traces of magnesium salts. Also phenolic impurities present in sodium salicylate alters its odour.

[GTU important]

# Sources of Impurities in Pharmaceuticals

**The type and amount of impurity present in the chemicals or pharmaceutical substances, depends upon several factors like those listed below:**

1) **Raw material used in manufacture**
2) **Reagents used in manufacturing process**
3) **Method/ process used in manufacture or method of manufacturing**
4) **Chemical processes used in the manufacture**
5) **Atmospheric contamination during the manufacturing process**
6) **Intermediate products in the manufacturing process**
7) **Defects in the manufacturing process**
8) **Manufacturing hazards**
9) **Inadequate Storage conditions**
10) **Decomposition of the product during storage**
11) **Accidental substitution or deliberate adulteration with spurious or useless materials**

## 1) Raw materials employed in manufacture

- Impurities known to be associated with these chemicals may be carried through the manufacturing process and contaminate the final product.
- Example

**Rock salt**--------→ Calcium Sulphate ($CaSO_4$) + Magnesium Chloride ($MgCl_2$)= NaCl prepared

Rock salt contains small amounts of ***Calcium sulphate and Magnesium chloride***. Thus Sodium chloride prepared from this source will contain traces of Calcium and Magnesium compounds.

Impurities such as Arsenic, Lead and Heavy metals are present in raw materials and hence are found in substances. So, it is necessary to use **pure chemicals** and substances as raw materials for the manufacturing process.

***Calcium sulphate and Magnesium chloride***

•**Example:**

- Copper sulphate may be prepared by the action of sulphuric acid on copper turnings:

$Cu + 2\ H_2SO_4 \longrightarrow CuSO_4 + 2\ H_2O + SO_2$

Copper turnings are known to have **Iron and Arsenic as impurities**.
If Large quantities of impurities are present in the raw material (e.g Copper turnings), they may enter the final product. ($CuSO_4.5H_2O$)

Due to this I.P. prescribes limit of tolerance for Arsenic as impurity to be not more than 8 parts per million in copper sulphate. Similarly it prescribes a limit of Iron as impurity.

**Copper turnings**

# 2) Reagents used in the manufacturing process:

- If reagents used in the manufacturing process are not completely removed by **washing**, these may find entry into the final products.

**Example:**

Ammoniated mercury may be prepared by adding a solution of Mercuric chloride to dilute ammonia solution.

$$HgCl_2 + 2NH_4OH \longrightarrow NH_2HgCl + NH_4Cl + 2H_2O$$

**Soluble** **soluble** **Ammoniated mercury (ppt)** **(soluble)**

The precipitate of Ammoniated mercury (Final Product)contains ammonium hydroxide. Thus, this precipitate is washed with cold water to remove ammonium hydroxide.

If it is not removed completely by washing with water, the final product may contain in it **Ammonium hydroxide as impurity**.

# 3) Method or the process used in the manufacture:

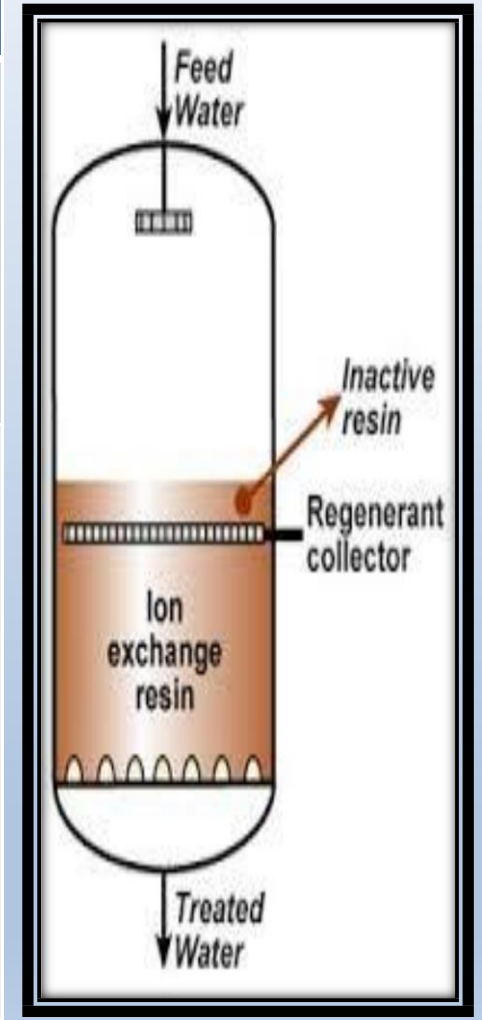
- Many drugs and chemicals (usually organic) are manufactured from different raw materials, by using different methods or processes.
- Some impurities are incorporated into the materials during the manufacturing process.
- The type and amount of impurity present in the drug/ chemical varies.
- In certain drugs , **a multiple-step-synthesis** procedure is used , which produces **intermediate compounds**.
- The purification of intermediates is also important, otherwise the impurities present in the intermediate will get incorporated in the final product.
- Usually **side reactions** occur during the synthesis.
- Impurities of the product side reactions also occur in the substances. This may introduce new impurities due to contamination by reagents and solvents at various stages of the process as described below:

a) **Reagents employed in the process**
b) **Reagents added to remove other impurities**
c) **Solvents**
d) **Action of solvents and reagents on reaction vessels.**

## C) Solvents:

Water is the cheapest solvent available and has been used wherever possible.

| Tap Water | It has $Ca^{+2}$, $Mg^{+2}$, $Na^{+}$, $Cl^{-}$, $SO_4^{-2}$ and $CO_3^{-2}$ as impurities in small amounts |
|---|---|
| **Softened water** | It is obtained by allowing the tap water to pass through the sodium form of Zeolite which removes divalent cations like $Ca^{+2}$ and $Mg^{+2}$ from tap water in exchange of sodium.<br>So, softened water contains Na+, $Cl^{-}$ ions as impurity. |
| **De-mineralised water** | It is obtained by passing tap water through columns packed with ion exchange resin.The water obtained from this process is free from $Ca^{+2}$, $Mg^{+2}$, $Na^{+}$, $Cl^{-}$, $SO_4^{-2}$ and $CO_3^{-2}$<br>Thus the final product is free from these impurities.<br><br>The water obtained from this source may still contain organic impurities and so final product contains organic impurities. |
| **Distilled water** | It is considered the best but it is very costly. |

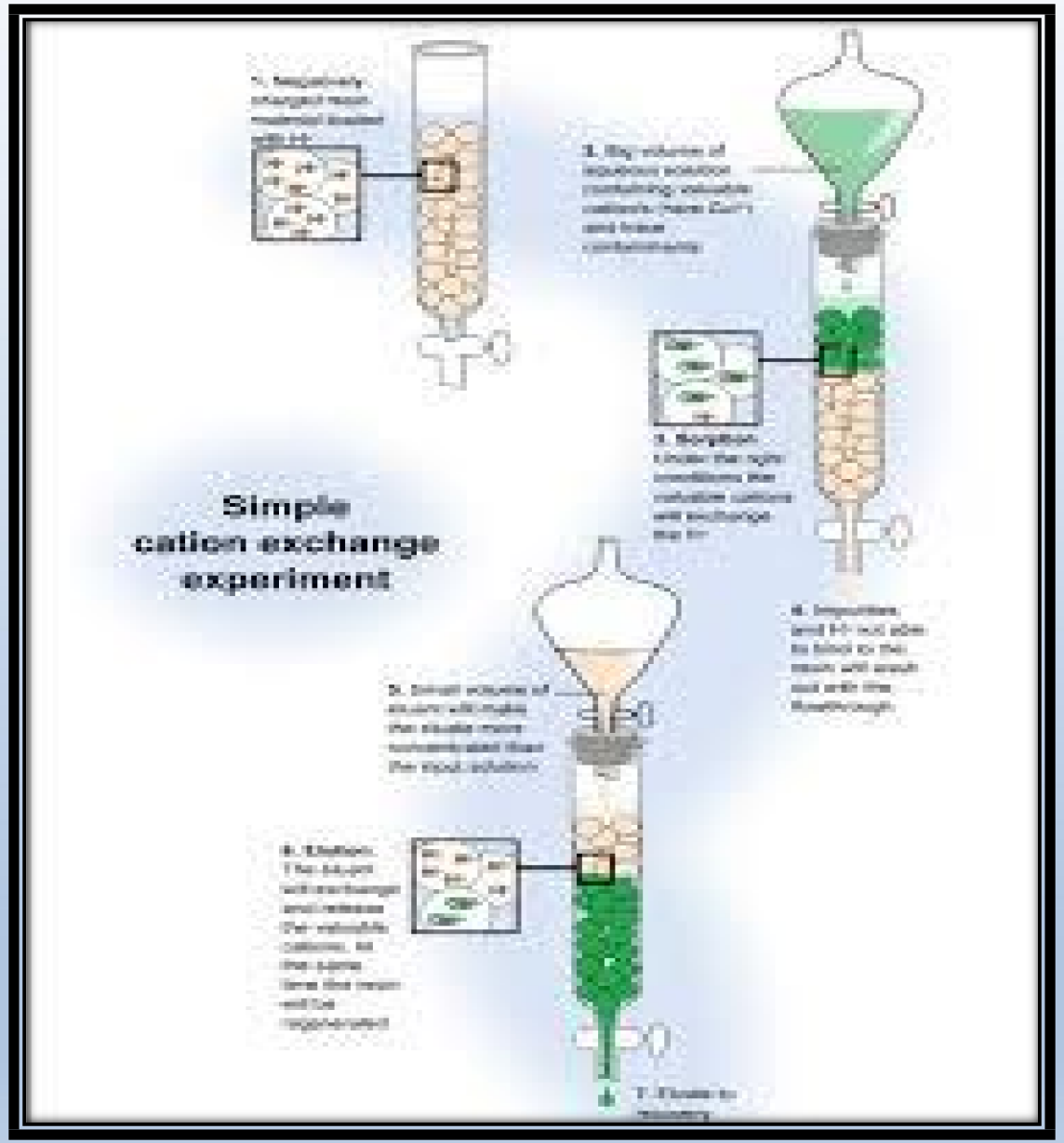


**a) Reagents employed in the manufacturing process:**

- Soluble alkali in Calcium carbonate arises from sodium carbonate used in the process.
- Calcium carbonate is obtained by interaction of a soluble calcium salt and a soluble carbonate and therefore the product will contain traces of soluble alkali, which the washing process has failed to remove.

**b) Reagents added to remove other impurities:**

- Potassium bromide contains traces of Barium, which is added in the manufacturing process to remove excess of sulphate.


Simple
cation exchange
experiment

## d) Action of solvents and reagents on reaction vessels:

During manufacturing process, some of the solvents and reagent may undergo reaction with metals of reaction vessel and may dissolve these metals, which appear as impurities in the final product.

**Example:**

- ✓ **Iron is known to contain Arsenic impurity.**
- ✓ The inorganic compounds manufactured in **Iron vessel** will contain Arsenic and Iron as impurities.
- ✓ Thus IP has prescribed limit test for Arsenic and Iron for most inorganic compounds.

**Iron vessel**

## 4) Chemical process used in the manufacture:

- For the synthesis of drugs, many chemical reactions such as Nitration, Halogenation, Oxidation, reduction, hydrolysis are involved.
- In these chemical processes, different chemicals are used.
- Tap water is generally used in the various processes and it is often having $Cl^{-}$,$Mg^{+2}$, $Ca^{+2}$ ions, which are generally found in the substance which is being manufactured.

# 5) Atmospheric contamination during the manufacturing process

- In the industrial areas, the atmosphere is contaminated with **dust particles** and some gases like **Hydrogen sulphide, Sulphur dioxide, and black smoke**.
- During the manufacture or purification of the pharmaceutical products, these impurities enter the final products.
- There are many pharmaceutical products which when manufactured are contaminated with atmospheric $CO_2$ and water vapour. E.g NaOH absorbs atmospheric $CO_2$.

- $2NaOH + CO_2 \dashrightarrow Na_2CO_3 + H_2O$

- Due to this reaction, NaOH should not be kept open for a longer time during its manufacture.

- Therefore, IP has prescribed that Sodium hydroxide should not contain more than 3% of sodium carbonate.

## 6) Defects in the manufacturing process:

In many manufacturing processes, there are defects like **imperfect mixing**, **incompleteness, non-adherence to proper temperature**, **pressure, pH or reaction conditions**, which may give chemical compounds with impurities in them.

**Example:**

- Zinc oxide may be prepared by heating metallic zinc to bright redness in a current of air. The vapours of Zinc burn to form Zinc oxide which is collected as a fine white powder.
- But if there is **less heat or air or both**, zinc metal is not completely converted to zinc oxide.
- Thus the final product, ***Zinc oxide may still contain metallic zinc as impurity.***
- So, IP has prescribed a test for Zinc metal in zinc oxide.

## 7) Intermediate products in the manufacturing process:

- There are some intermediates which are produced during the manufacturing process. Sometimes these intermediates may be carried through to the final product as impurity.
- Example:

Potassium iodide is prepared by reacting Iodine with Potassium hydroxide.

$6KOH + 3I_2 \text{----------} \rightarrow 5KI + KIO_3 + 3H_2O$

The resulting solution is first evaporated and then heated with charcoal.

$KIO_3 + 3C \text{----------} \rightarrow KI + 3CO$

- In this process if the intermediate product ($KIO_3$) is not completely converted into KI, then it may be carried through to the final product as an impurity.

# 8) Manufacturing hazards:

- **Particulate contamination**
- Process errors
- **Cross contamination**
- **Microbial contamination**
- Packing errors

# Particulate contamination:

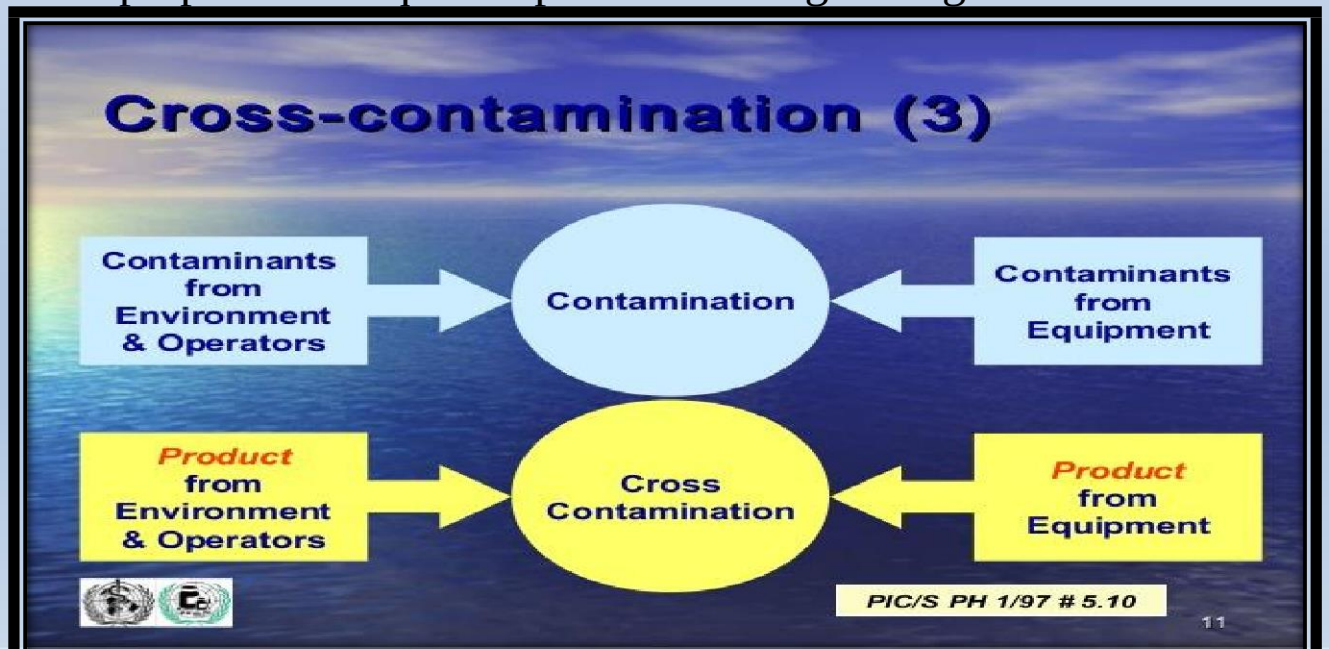
- The presence of unwanted particulate matter can arise due to dirt, dust, glass, porcelain or plastic fragments from sieves, granulating or tableting machines or from product containers.
- Ware and tare of equipment or improperly cleaned equipment may also cause particulate contamination.
- Clarity of solutions for injection is particularly important.
- E.g Metal particles which have been found in eye ointments packed in metal tubes.


## Process errors:

- Gross errors arising from incomplete solution of a solute in a liquid preparation must be detected readily by the normal analytical control procedures.
- Minor errors arise if the manufacturing tolerance for the quantity of active ingredient in the product has been wide.

## Cross contamination:

- The handling of powders, granules, and tablets in large bulk creates air-borne dust, which leads to cross contamination of the product.
- So, face masks and special extraction equipment are used to protect operators from harmful effects of drugs.
- E.g penicillin preparation requires special handling during its manufacture.



# Microbial contamination:

- ***Parenteral preparations*** and ***ophthalmic preparations*** require special care against microbial contamination.
- Many liquid preparations and creams are liable to bacterial and fungal contamination. So care should be taken.

- Eg. Acacia, senna, tragacanth---→They should be controlled for Salmonellae.

# Packing errors:

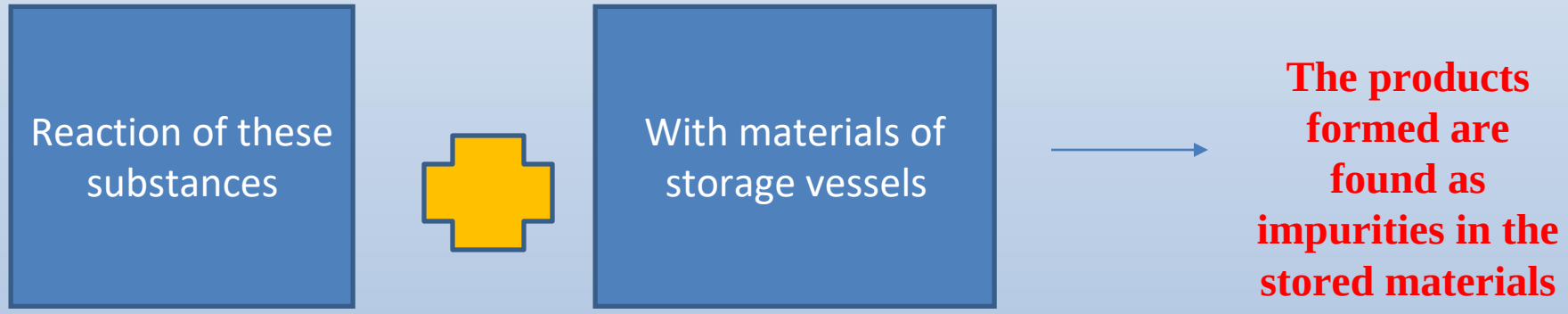
- Products of similar appearance such as tablets of same size, shape, colour packed in similar containers can constitute a potential source of danger.
- Improper labelling or destruction of stock of unused labels also constitutes a major packaging hazard.

## 9) Storage conditions:

- The chemical substances when prepared have to be stored in different types of containers depending upon:

✓ Nature of the material
✓ Batch size
✓ Quantity

- Many types of materials are used for storage purpose like plastic, polythene, iron vessels, stainless steel and aluminium.



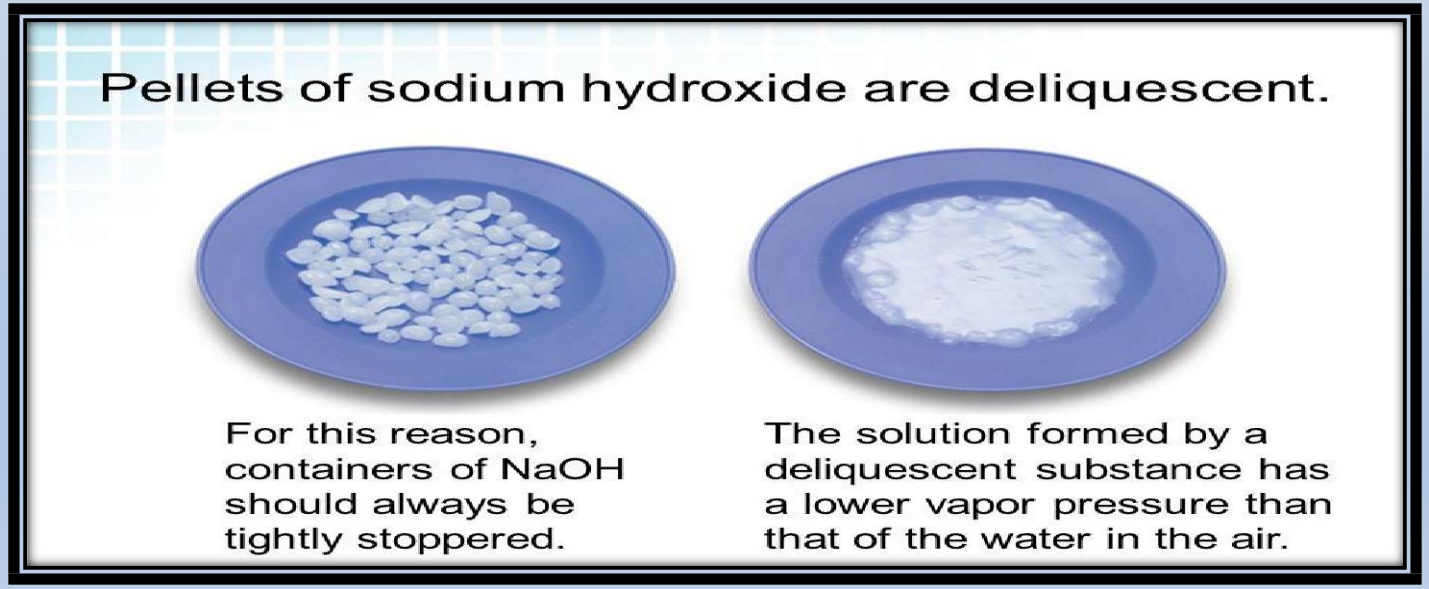

- **Leaching out effect:** Alkalies stored in ordinary glass containers extract lead from it, which in found as impurity in the final product.
- Strong chemicals react with iron containers and extract Iron an impurity in final product.

**Inadequate storage and their effects are as follows:**

a) **Filth:** Stored products may become contaminated with dust, bodies of insects, animal and insect excreta.
b) **Chemical instability:** decomposition because of light, traces of acid or alkali, air oxidation, water vapour, CO2 and traces of metallic ions.
   e.g light sensitive materials should be stored in amber colored bottles.
c) **Reactions with container materials:** e.g salicylic acid ointment must not be stored in metal tubes.
d) **Physical changes:** The occurance of changes in the physical form of drug like change in crystal size can lead to change in efficiency of product.
e) **Temperature effect:** Chemical and physical changes occur if materials are not stored at proper temperature.

# 10) Decomposition of the product during storage:

- **Chemical decomposition**, **analysis** or **breakdown** is the separation of a chemical compound into elements or simpler compounds. It is sometimes defined as the exact opposite of a chemical synthesis. Chemical decomposition is often an undesired chemical reaction.
- **Some substances decompose on storing due to presence of air, light and oxygen. So, the final product is contaminated.**
- **Deliquescent substances, absorb water from the atmosphere and get liquefied.**
- **Decomposition products appear as impurities in the substances.**



## 11) Accidental substitution or deliberate adulteration with spurious or useless materials:

- It is possible to avoid accidental substitution by storing the toxic substances together separately or in a locked cupboard.

- Many pharmaceutical chemicals are adulterated with cheaper substances.

- E.g The expensive potassium may be adulterated with sodium bromide.

# Effect of Impurities:

The impurities present in the substances may give following effects:

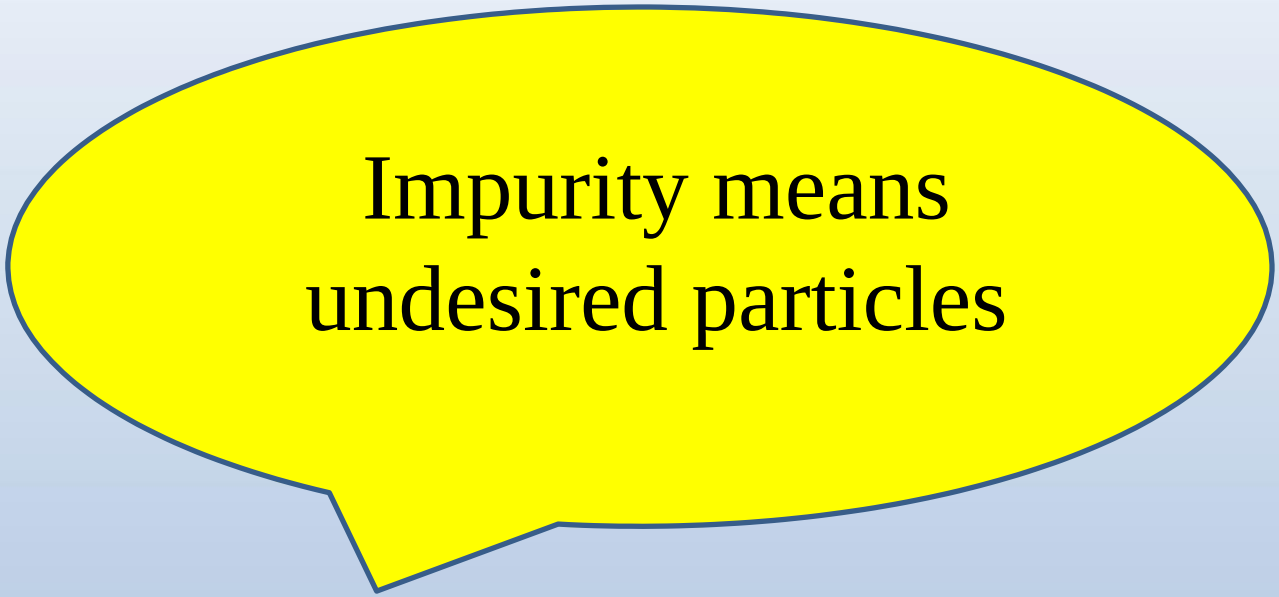
- Impurities having toxic effects may be injurious to health, if present above certain limits.
- Traces of impurities, may exert a cumulative toxic effect after a certain time.
- Impurities may lower the active strength of the substance.
- Impurity may decrease shelf life of substance.
- Impurity may cause incompatibility with other substances.
- Impurities may cause a physical or chemical change in the properties of the substance, so making the substance medicinally useless.
- May cause change in color, odour and taste.

## Test for purity:

- ❖ Pharmacopoeia prescribes the “Test for purity” for pharmaceutical substances to check their freedom from undesirable impurities.
- ❖ Pharmacopoeia will decide and fix the limit of tolerance for these impurities.
- ❖ For certain common impurities for which pharmacopoeia prescribes the test of purity are:
- ✓ Colour, odour, taste
- ✓ Physicochemical constants (Iodine value, saponification value, melting point, refractive index etc.)
- ✓ Acidity, alkalinity, pH
- ✓ Humidity (Estimation of moisture)
- ✓ Cations and anions
- ✓ Ash
- ✓ Arsenic or lead
- ✓ Loss on drying
- ✓ Loss on ignition

**What is impurity?**

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

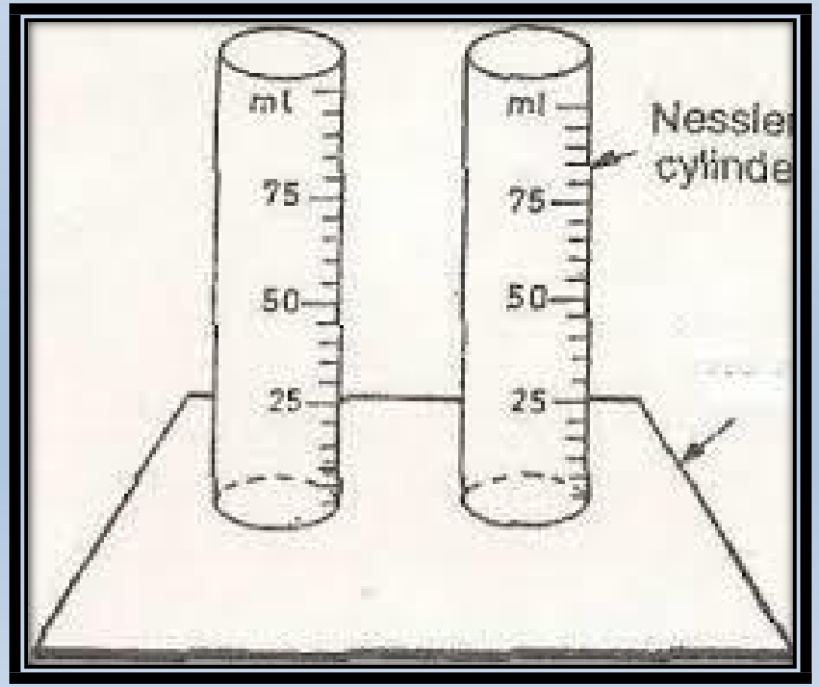



- ***Presence of Impurities in the pharmaceutical substances may produce toxic effects on the body and may also lower down the active strength of the pharmaceutical substance.***
- ***Impurities commonly in chemical substances include small quantities of lead, Arsenic, Chloride and sulphate.***

# 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

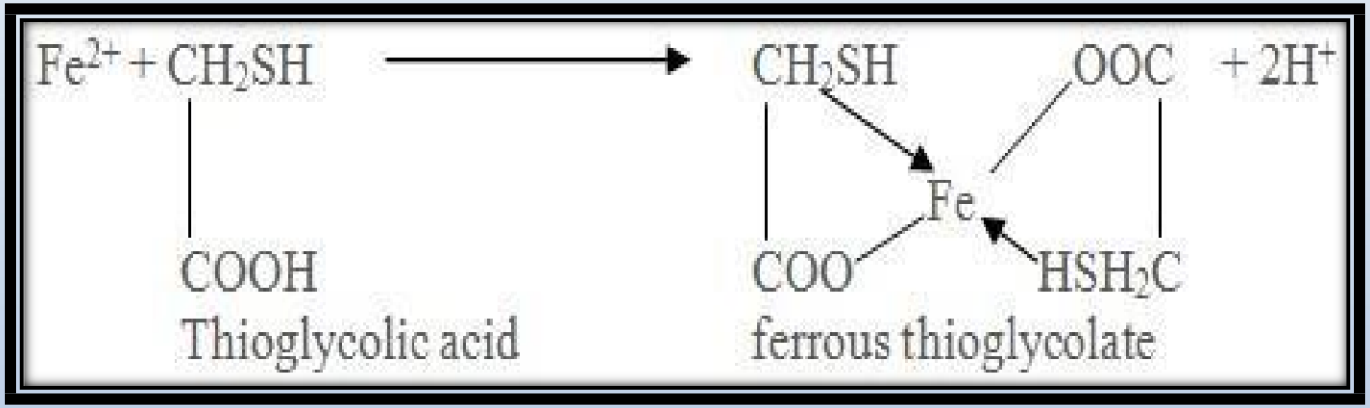
# Types:

- **Tests in which there is no visible reaction**
- **Comparison methods**
- **Quantitative determinations**

# Turbidity means cloudiness or haziness.

# 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.




- The color of the Ferrous thioglycolate complex fades in the presence of air due to oxidation.
- Also, the color is destroyed in presence of oxidizing agents and strong alkalis.
- The **purple color is developed only in alkaline media**. **So ammonia solution is used.**
- But ammonia reacts with iron, forms precipitate of **ferrous hydroxide**.
- Thus citric acid is used which prevents the precipitate of iron with Ammonia by forming a complex with iron as iron citrate.

# 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 |

**Note: All the reagents used in the limit test for Iron should themselves be iron free.**

**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 Chloride:

**The test is used to limit the amount of Chloride as an impurity in inorganic substances.**

# 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.

$Cl^- + AgNO_3 \longrightarrow AgCl + NO_3^-$

**Soluble chloride present as impurity**

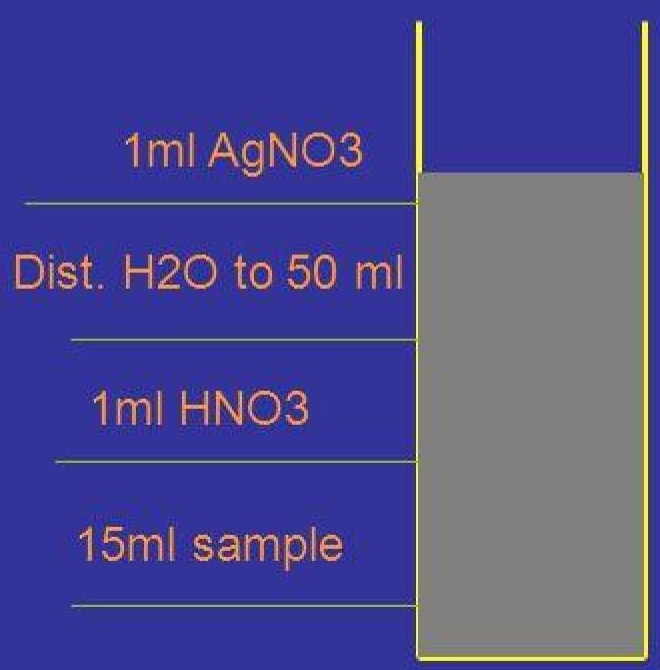
**silver chloride**

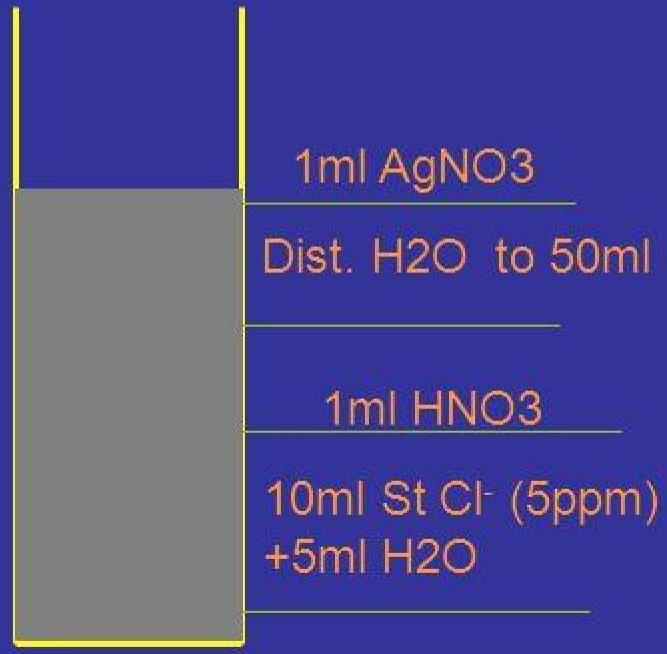
❖ The silver chloride produced in the presence of dilute Nitric acid makes the test solution turbid, the extent of turbidity depending upon the amount of Chloride present in the substance is compared with the standard opalescence produced by the addition of Silver nitrate to a standard solution having a known amount of chloride and the same volume of dilute nitric acid as used in the test 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.

- Pharmacopoeia does not prescribe any numerical value of limit test for chlorides, sulphate and iron because limit test is based on the simple comparison of opalescence or colour between the test and standard solution prescribed according to pharmacopoeia.
- In this type of limit test, the extent of turbidity or opalescence or colour produced in influenced by the presence of other impurities present in the substance and also by variation in time and method of performance of test.
- Thus the pharmacopoeia does not prescribe any numerical value of the limit test.

*Pharmacopoeia not prescribe any numerical value for limit test for chlorides, sulphate and iron because limit test is based on simple comparison of opalescence or color between test and standard solution prescribed according to pharmacopoeia. The variation in the permissible limits for various substances is obtained by taking varying quantities of the substances under test. In this type of limit test, the extent of opalescence or turbidity or color produced is influenced by the presence of other impurities present in the substance and also by variation in time and method of performance of tests and hence the pharmacopoeia do not prescribe any numerical values for the limit test in these test.*

- The limit test involve simple comparisons of opalescence, turbidity, or colour with standard.
- These are semi-qualitative reactions in which extent of impurities present can be estimated by comparing visible reaction response of the test and standard.
- By this way, extent of reaction is readily determined by direct comparison of test solution with standard. So pharmacopoeia prefers comparison methods.

# 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.


**$SO_4^{-2}$ + BaCl $_2$----------→ BaSO $_4$ + $2Cl^-$**

Then comparison of turbidity is done with a standard turbidity obtained from a known amount of Sulphate and same volume of dilute Hydrochloric acid have been added to both solutions. The 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.

**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 |

Barium sulphate reagent contains barium chloride, sulphate free alcohol and small amount of potassium sulphate.

## 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.

# Limit test for Arsenic:

- Arsenic is a well known undesirable and harmful impurity which is present in medicinal substances.
- All pharmacopoeias prescribe a limit test for it.
- Pharmacopoeial method is based on the **Gutzeit test.**

- All the special reagents used in the limit test for Arsenic are marked and distinguished by letter ‘As T’, which means that they all should be Arsenic free and should themselves conform to the test for Arsenic.

## Principle:

Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form **yellow stain** on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as **Gutzeit test** and requires special apparatus.

- Arsenic, present as arsenic acid ($H_3AsO_4$) in the sample is reduced to arsenious acid ($H_3AsO_3$) by reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid, etc. Arsenious acid is further reduced to arsine (gas) ($AsH_3$) by hydrogen and reacts with mercuric chloride paper to give a yellow stain.

- **Substance + dil HCl ----------→ $H_3AsO_4$**
  **(contains Arsenic impurity) Arsenic acid**

- **$H_3AsO_4$ + $H_2SnO_2$ ----------→ $H_3AsO_3$ + $H_2SnO_3$**
  **Arsenic acid Arsenious acid**

  **$H_3AsO_3$ + 6[H] ----------→ $AsH_3$ + $3H_2O$**
  **Arsenious acid nascent hydrogen Arsine gas**

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

- When the sample is dissolved in **acid**, the Arsenic present in the sample gets converted to **Arsenic acid.**
- By action of reducing agents like Potassium iodide, stannous acid etc., Arsenic acid gets reduced to **arsenious acid**.
- The **nascent hydrogen** formed during the reaction, further reduces **Arsenious acid** to **Arsine gas**, which reacts with mercuric chloride paper, giving a yellow stain.

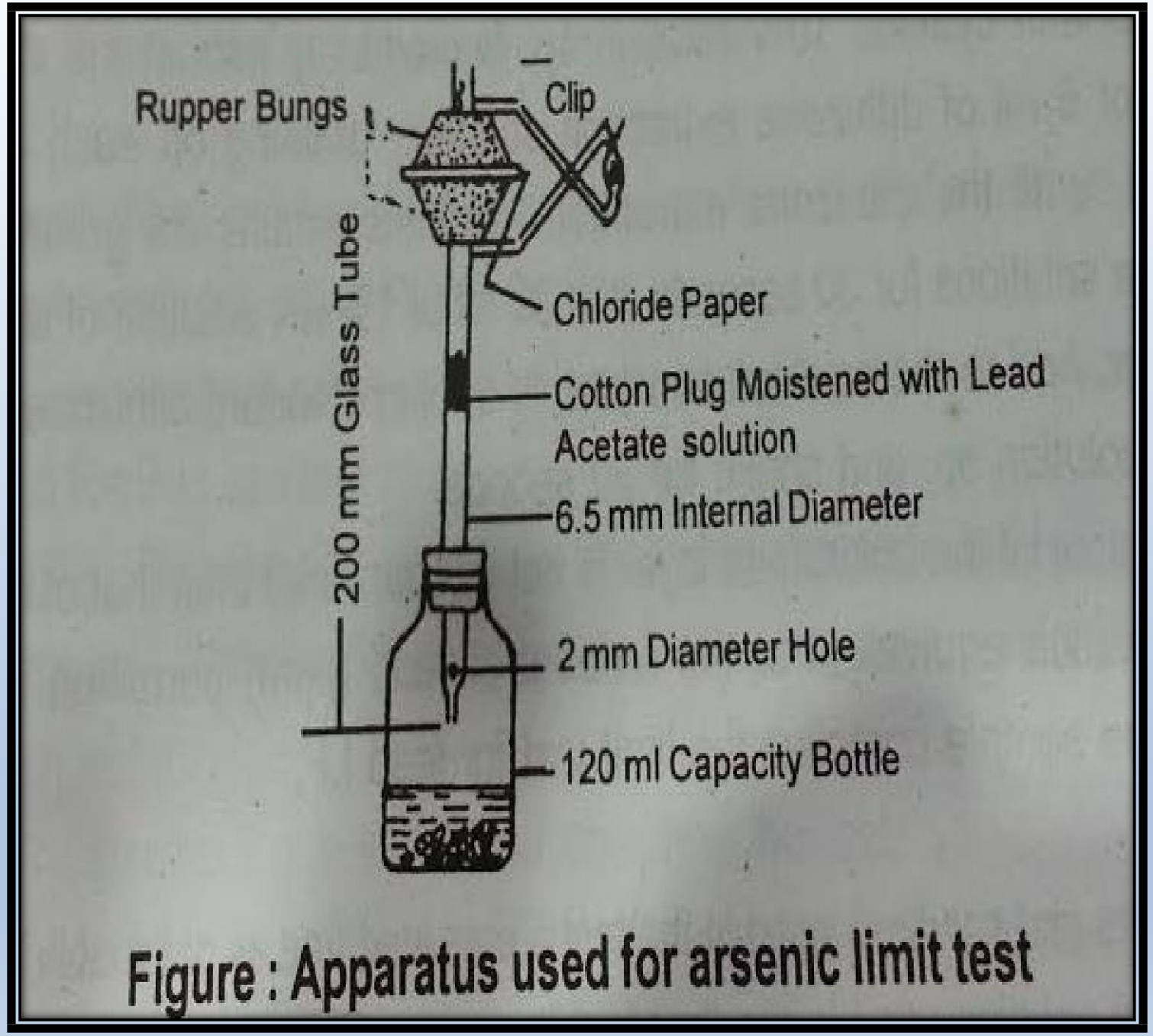



Figure : Apparatus used for arsenic limit test

## Apparatus:

- ✓ It is having a wide mouthed glass bottle of 120 mL capacity having mouth of about 2.5 cm in diameter. This bottle is fitted with a rubber bung through which passes a glass tube, 20 cm long.
- ✓ External diameter=0.8 cm
- ✓ Internal diameter=0.65 cm
- ✓ 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.
- ✓ The upper end of the glass tube is fitted with two rubber bungs(25 mm x 25 mm), each having a hole bored centrally and exactly 6.5 mm in diameter.
- ✓ 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.
- ✓ The bungs are kept in close contact by using rubber band or spring clip in such a manner that the gas evolved from the bottle must have to pass through the 0.65 mm internal circle of mercuric chloride paper.
- ✓ 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.
- ✓ An important feature has been the standardization of the area of Mercuric chloride paper which is exposed to the reaction of arsine gas.

| Test sample | Standard compound |
| --- | --- |
| The test solution is prepared by dissolving specific amount in water and stannated HCl (arsenic free) and kept in a wide mouthed bottle. | A known quantity of dilute arsenic solution in water and stannated HCl (arsenic free) is kept in wide mouthed bottle. |
| 1 g of KI | 1 g of KI |
| 5 ml of stannous chloride acid solution | 5 ml of stannous chloride acid solution |
| 10 g of granulated zinc is added (all this reagents must be arsenic free).<br><br>Keep the solution aside for 40 min | 10 g of zinc is added (all this reagents must be arsenic free).<br><br>Keep the solution aside for 40 min |

Stain obtained on mercuric chloride paper is compared with standard solution. Standard stain must be freshly prepared as it fades on keeping.

**Inference:** If the stain produced by the test is not deeper than the standard stain, then sample complies with the limit test for Arsenic.

**Reasons:**

Stannous chloride is used for complete evolution of arsine.

Zinc, potassium iodide and stannous chloride is used as a reducing agent.

Hydrochloride acid is used to make the solution acidic

**Lead acetate paper** are used to trap any hydrogen sulphide which may be evolved along with arsine.

**Use of stannated Hydrochloric acid:**

If pure zinc and HCl are used, the steady evolution of gas does not occur. This produces improper stain (e.g slow evolution produces short but intense stain while rapid evolution of gas produces long but diffused stain.)

So, to get steady evolution of gas, stannated hydrochloric acid is used.

**Use of Lead Acetate solution:**

$H_2S$ gas may be formed during the experiment as zinc contains sulphides as impurities. It gives black stain to $HgCl_2$ paper and so will interfere the test.

Hence, gases evolved are passed through cotton wool plug moistened with lead acetate, where $H_2S$ gas is trapped as PbS.

**Use of Potassium iodide:**

KI is converted to HI which brings about reduction of unreacted pentavalent arsenic to trivalent Arsenic. Thus, reproducible results can be obtained. If it is not used then some pentavalent Arsenic may remain unreacted.

# Limit test for heavy metals

- 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 should not exceed the heavy metal limits given under the individual monograph.
- The heavy metals (metallic impurities) may be iron, copper, lead, nickel, cobalt, bismuth, antimony etc.. **The limit for heavy metals is indicated in the individual monograph in term of ppm of lead i.e. the parts of lead per million parts of the substance being examined**
- In substances the proportion of any such impurity (Heavy metals) has been expressed as the quantity of lead required to produce a color of equal depth as in a standard comparison solution having a definite quantity of lead nitrate.
- The quantity is stated as the heavy metal limit and is expressed as parts of lead (by weight) per million parts of the test substance.
- **The limit test for heavy metals has been based upon the reaction of the metal ion with hydrogen sulphide, under the prescribed conditions of the test causing the formation of metal sulphides.**

- These remain distributed in colloidal state, and give rise to a brownish coloration.

- I.P limit for heavy metals in 20 ppm.
- The test solution is compared with a standard prepared using a lead solution (as the heavy metal).The metallic impurities in substance are expressed as parts of lead per million parts of substance.
- IP has adopted 3 methods for this:

- **Method I**: The method is applicable for the samples which give clear colourless solutions under specified conditions of test.
- **Method II**: The method is applicable for the samples which DO NOT give clear colourless solutions under specified conditions of test.
- **Method III**: Used for substances which give clear colourless solutions in sodium hydroxide medium.

## 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 diphenyl thiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is red in color.

- Dithizone in chloroform, is able to extract lead from alkaline aqueous solutions as a lead dithizone complex (Red in colour)
- The original dithizone is having a green colour in chloroform while the lead-dithizone is having a violet color.So, resulting color at the end of the process is read.
- **The intensity of the color of complex is dependant upon the amount of lead in the solution.**
- The color of the lead-dithizone complex in chloroform has been compared with a standard volume of lead solution, treated in the same manner.
- In this method, the lead present as an impurity in the substances, gets separated by extracting an alkaline solution with a dithizone extraction solution.
- **The interference and influence of the other metal ions has been eliminated by adjusting the optimum pH for the extraction by employing Ammonium citrate/ potassium cyanide.**

Method:

- Sample solution is transferred to a separating funnel.
- To it 6 ml of ammonium citrate, 2 ml potassium cyanide and 2 ml of hydroxalamine HCl are added.
- 2 drops of phenol red
- Solution is made alkaline by adding ammonia solution.
- This is then extracted with 5 ml portions of dithizone solution until it becomes green.
- The combined dithizone extracts are shaken for 30 seconds with 30 ml of nitric acid and chloroform layer is discarded.
- To the acid solution 5 ml of standard dithizone solution is added and 4 ml ammonium cyanide and solution is shaken for 30 sec.
- Similarly prepare standard.

- **Observation:**

  The intensity of the color of complex, is depends on the amount of lead in the solution. The color produced 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 by extracting an alkaline solution with a dithizone extraction solution.

| **A known quantity of sample solution is transferred in a separating funnel** | **A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination** |
|---|---|
| Add 6ml of **ammonium citrate** | Add 6ml of ammonium citrate |
| Add 2 ml of **potassium cyanide** and 2 ml of **hydroxylamine hydrochloride** | Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride |
| Add 2 drops of phenol red | Add 2 drops of phenol red |
| Make solution alkaline by adding ammonia solution. | Make solution alkaline by adding ammonia solution. |
| Extract with 5 ml of **dithizone** until it becomes green | Extract with 5 ml of dithizone until it becomes green |
| Combine dithizone extracts are shaken for 30 mins with 30 ml of **nitric acid** and the chloroform layer is discarded | Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded |
| To the acid solution add 5 ml of standard dithizone solution | To the acid solution add 5 ml of standard dithizone solution |
| Add 4 ml of ammonium cyanide | Add 4 ml of ammonium cyanide |
| Shake for 30 mins | Shake for 30 mins |
| Observe the color | Observe the color |

## Aq. Ammonia is added in limit test of lead:

- Pb+ S---------- →PbS
- In limit test of lead, PbS is produced by addition of standard $H_2S$ , to the solution containing lead.
- pH 3-4 is necessary for the precipitation of PbS. So aq. Ammonia / acetic acid is added to maintain that pH.

# Modified limit test for Chlorides

Depending upon the nature of the substance, some modifications have to be adopted for the preparation of the solution.

# Modified limit test for Chlorides

(a) Alkaline substances have to be dissolved in acid so that effervescence ceases and much of the free acid is left in the solution as is prescribed in the test.

(b) Insoluble substances are generally extracted with water and then filtered, and the filtrate is used for the test, because the presence of insoluble substance modifies the opalescence and colour.

(c) Salts of organic acids like sodium benzoate, sodium salicylate, etc. liberate free water insoluble organic acid during acidification which is filtered off and the filtrate is employed for the test.

(d) Coloured substances like crystal violet, malachite green, etc. are carbonised and the ash so produced is extracted in water.

(e) Deeply coloured substances have to be decolourised before test e.g., potassium permanganate is reduced by boiling with alcohol and the filtrate is used.

(f) Reducing substances like hypophosphorus acid, which react with silver nitrate in the limit test for chlorides should be oxidized with nitric acid or some other oxidizing agents before carrying out the test.

# Modified limit test for Chlorides

**Aim** To perform the limit test for chloride in potassium permanganate sample (according to IP'96)

**Requirement:**

Nessler's cylinder, measuring cylinder, pipette, spatula, distilled water, dilute nitric acid, 0.1 M silver nitrate solution, potassium permanganate sample

**Principle:**

The limit test for chloride based on the reaction between soluble chloride impurities present in the substance and silver nitrate solution to give white precipitates of silver chloride. These white precipitates are insoluble in dilute nitric acid and hence give turbidity or opalescence to the test solution. The extent of the turbidity produced depends upon the amount of the chloride present in the substance which is compared with a standard opalescence produce by addition of silver nitrate to a standard solution having known amount of chloride and the same volume of the dilute nitric acid as the use in the test solution. If the turbidity developed in the sample is less than the standard turbidity, the sample passes the limit test for chloride and vice-versa. **As potassium permanganate gives purple color aqueous solution that interferes in the comparison of opalescence or turbidity, therefore the aqueous solution must first be decolorized. Potassium permanganate is oxidizing agent while ethanol is reducing agent.**

**When potassium permanganate solution is treated with ethanol in presence of heat the redox reaction will take place, i.e. potassium permanganate gets reduced to manganese dioxide (precipitates). The filtrate of the reaction is colorless that is subjected to proceed for limit test for chloride.**

**Chemical Reaction:**

$$2\ KMnO_4 + 3\ C_2H_5OH \xrightarrow{\longrightarrow} 2\ MnO_2 + 2\ KOH + 2\ CH_3CHO + 2\ H_2O$$

| Sr. No. | STANDARD SOLUTION | SR. NO | TEST SOLUTION |
|---|---|---|---|
| 1 | Take 10 ml chloride standard solution (25 ppm chloride) and add 5 ml water in a Nessler's cylinder. | 1 | **Transfer the prepared test solution in Nessler's cylinder** |
| 2 | Add 10 ml of dilute nitric acid and dilute to 50 ml with distilled water | 2 | **Add 10 ml of dilute nitric acid and dilute to 50 ml with distilled water** |
| 3 | **Add 1ml of 0.1 M silver nitrate solution and stir immediately with glass rod and allow standing for 5 minutes protected from light.** | 3 | **Add 1ml of 0.1 M silver nitrate solution and stir immediately with glass rod and allow standing for 5 minutes protected from light.** |

Compare the turbidity or opalescence produced in test solution with respect to standard solution and report the result and conclusion.

**Observation and conclusion:**

**Observation and conclusion will be of two types:**

If the intensity of turbidity or opalescence appears to be more in test solution than the standard solution then conclusion is impurities of chloride in given sample is over the limit as per IP’96. Hence, sample do not passes the limit test for chlorides.

If the intensity turbidity or opalescence appears to be less or equal in test solution than the standard solution then conclusion is impurities of chloride in given sample is under the limit as per IP’96. Hence, sample passes the limit test for chloride

# To perform the limit test for sulphate in Potassium permanganate sample (According to IP'96)

**Principle:**

It is a comparison method. It involves the comparison of opalescence or turbidity of test sample verses standard sample which contain the definite amount of sulphate impurities.

The limit test of sulphate is performed on the basis of reaction between the barium chloride reagent (containing barium chloride, sulphate free alcohol and solution of potassium sulphate ($K_2SO_4$) and soluble sulphate in the sample with formation of barium sulphate ($BaSO_4$) white precipitates.

Sulphate free alcoholic potassium sulphate is added to increase the sensitivity of the test. Very small amount of barium sulphate present in the reagents acts as a seeding agents for precipitation of barium sulphate, if sulphate is present in the sample under the test.

Ethanol is added to prevent the super saturation i.e. the crystallization of sulphate with any other ion.

As potassium permanganate gives purple colored aqueous solution that interferes in the comparison of opalescence or turbidity, therefore it requires to be decolorized. Potassium permanganate is oxidizing agent while ethanol is reducing agent. When potassium permanganate solution is treated with ethanol in presence of heat the redox reaction takes place, i.e. potassium permanganate gets reduced to manganese dioxide (precipitates) and ethanol gets oxidized to form ethanal. The filtrate of the reaction is colorless that is subjected to proceed for limit test for sulphate.

**Reaction:**

$$2\ KMnO_4 + 3\ C_2H_5OH \text{----------} \rightarrow 2\ MnO_2 + 2\ KOH + 2\ CH_3CHO + 2\ H_2O$$

| Sr. No. | STANDARD SOLUTION | SR. NO | TEST SOLUTION |
|---|---|---|---|
| 1 | Take 1 ml 25% w/v barium chloride in Nessler's cylinder and add 1.5 ml of ethanolic sulphate standard solution (10 ppm $SO_4^{-2}$). Mix and allow to stand for 1 minutes | 1 | **Take 1 ml 25% w/v barium chloride in Nessler's cylinder and add 1.5 ml of ethanolic sulphate standard solution (10 ppm $SO_4^{-2}$). Mix and allow to stand for 1 minutes** |
| 2 | Add 15 ml of standard sulphate solution (10 ppm $SO_4^{-2}$) and 0.15 ml of 5M acetic acid. | 2 | **Transfer prepared test solution and add 0.15 ml of 5 M acetic acid.** |
| 3 | **Add sufficient distilled water to produced 50 ml. Stirred it immediately and allow standing for 5 minutes.** | 3 | **Add sufficient distilled water to produced 50 ml. Stirred it immediately and allow standing for 5 minutes.** |

- Compare the turbidity or opalescence in the test solution by viewing transversely both solutions against black background.
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