

UNIT-I

Limit Tests

Limit Test: Limit tests are quantitative or semi-quantitative tests designed to identify and control small quantities of impurities, which are likely to be present in the pharmaceutical substance

Impurity: The impurities in pharmaceuticals are the unwanted foreign chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products

Limit = a value or amount that is likely to be present in a substance

Test = to examine or to investigate

A limit test is defined as a quantitative or semi-quantitative test designed to identify and control small quantities of impurity which is likely to be present in the substance

A limit test is generally carried out to determine the inorganic impurities present in compound. In short, the limit test is nothing but to identify the impurities present in the substance and compare it with standard

Importance of Limit tests:

To find out the harmful amount of impurities
To find out the avoidable/unavoidable amount of impurities

TYPES OF INORGANIC IMPURITIES

1. Toxic impurities: This kind of impurities are very harmful and can even cause death when taken once or short period of time. Example: Arsenic

- 2. Cumulative impurities:** This kind of impurities shows their toxicity when it taken over a period of time. Example: Heavy Metals
- 3. Harmless impurities:** Some impurities may not cause harm to body but reduce the therapeutic activity of active ingredient when it is present in large quantities. Example: Chlorides, Sulphates.

On considerations of above classification, the pharmacopoeia has fixed the permissible limits of each impurity. For toxic impurities the permissible limit is as less as 5-10 ppm, whereas for cumulative impurities the permissible limit is 20 ppm. For harmless impurities the limits are still high.

Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now getting receiving important critical attention from regulatory authorities because practically it is impossible to remove all the impurities from any pharmaceuticals. Some remain in trace even after purification, so it is only desirable that the substance should be sufficiently pure and can be used safely. The different pharmacopoeias (IP, BP, USP etc.) specify the limits up to which various impurities can be tolerated in pharmaceuticals. The limit tests help to check and indicate the presence of various inorganic impurities in pharmaceuticals.

Obviously the quantity of any impurity present in official pharmaceuticals is often small, and therefore, the normal visible-reaction-response to any test for that impurity is also quite small. Hence, it is essential to design the individual test in such a manner so as to avoid possible errors in the hands of various analysts. This is accomplished by taking into consideration the following three cardinal factors

- 1. Specificity of the tests:** Any test used as a limit test must, of necessity, give some form of selective reaction with the trace impurity. Many tests used for the detection of inorganic impurities in official inorganic chemicals are based upon the separations involved in inorganic qualitative analysis. A test may be demanded which will exclude one specific impurity, but highly specific tests are not always the best; a less specific test, which limits several likely impurities, at once, is obviously advantageous, and in fact can often be accomplished. An example of such a test is the heavy metals test applied to alum, which not only limits contamination by lead, but also other heavy metal contaminants precipitated by thioacetamide as sulphide at pH 3.5.
- 2. Sensitivity:** The degree of sensitivity required in a limit test varies enormously according to the standard of purity demanded by the monograph. The sensitivity of most tests is dependent upon a number of variable factors all capable of strict definition, and all favorable towards the production of reproducible results. Thus the precipitation of an insoluble substance from solution is governed by such factors as concentration of the solute and of the precipitating reagent, duration of the reaction, reaction temperature, and the nature and concentration of other substances unavoidably present in solution. As a general rule, cold dilute solutions give light precipitates, whereas more granular ones are obtained from hot concentrated solutions. Many of the limit tests, however, are concerned with very dilute solutions, which are often slow to react, and here sensitivity of the reaction can often be increased by extending the duration of the reaction or by raising the reaction temperature. Similar considerations apply in the design of colour and other tests employed as limit tests.
- 3. Control of personal errors:** It is essential to exclude all possible sources of ambiguity in the description of a test. Vague terms such as 'slight precipitate,' should be avoided as far as possible. The extent of the visible reaction to be expected under the specified test

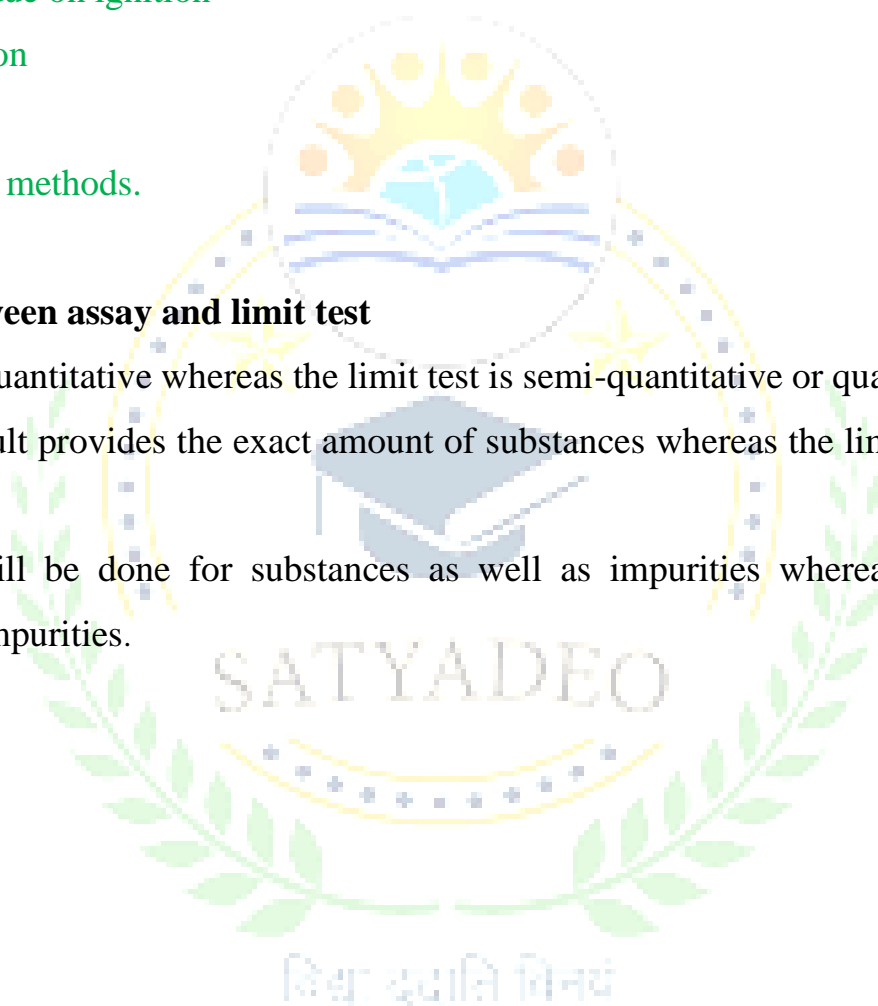
conditions should be clearly and precisely defined. This is usually accomplished in one of three ways.

- (a) **Tests in which there is no visible reaction:** A definite statement is incorporated in the wording of the test, which states that there shall be no colour, opalescence or precipitate, whichever is appropriate to the particular test. One example of this type of requirement is the test for barium, and calcium in dilute hypo-phosphorus acid, where the additions of dilute sulfuric acid under precisely controlled condition shall produce 'no turbidity, or precipitate' within one hour. The time factor is used here as a means of increasing the sensitivity of the test. Tests such as these which give negative results do not necessarily imply the complete absence of the impurity, the test as laid down merely indicating the absence of an undesirably large amount of the impurity
- (b) **Comparison methods:** Tests of this type require a standard containing a definite amount of impurity, to be set up at the same time and under the same conditions as the test experiment. In this way the extent of the reaction is readily determined by direct comparison of the test solution with a standard of known concentration. The official limit tests for chlorides, sulfates, iron and heavy metals are based on this principle. The limit tests for lead and arsenic are, in practice, also comparison methods. They are, however, so designed that they can be readily applied as quantitative determinations.
- (c) **Quantitative determinations:** Quantitative determination of impurities is only applied in special circumstances, usually in those cases where the limit is not readily susceptible to simple and more direct chemical determination. The method is used in the following different types of tests:

- i. Limits of insoluble matter
- ii. Limits of soluble matter
- iii. Limits of moisture, volatile matter, and residual solvents
- iv. Limits of non-volatile matter
- v. Limits of residue on ignition
- vi. Loss on ignition
- vii. Ash values
- viii. Precipitation methods.

Differences between assay and limit test

1. The assay is quantitative whereas the limit test is semi-quantitative or qualitative test.
2. The assay result provides the exact amount of substances whereas the limit tests for range of impurities.
3. The assay will be done for substances as well as impurities whereas the limit tests particularly for impurities.



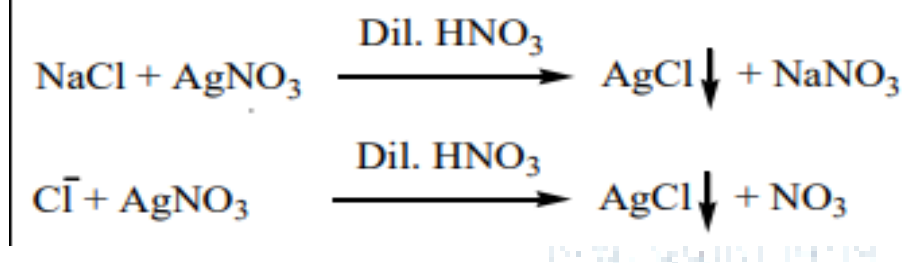
Limit Test for Chloride

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.

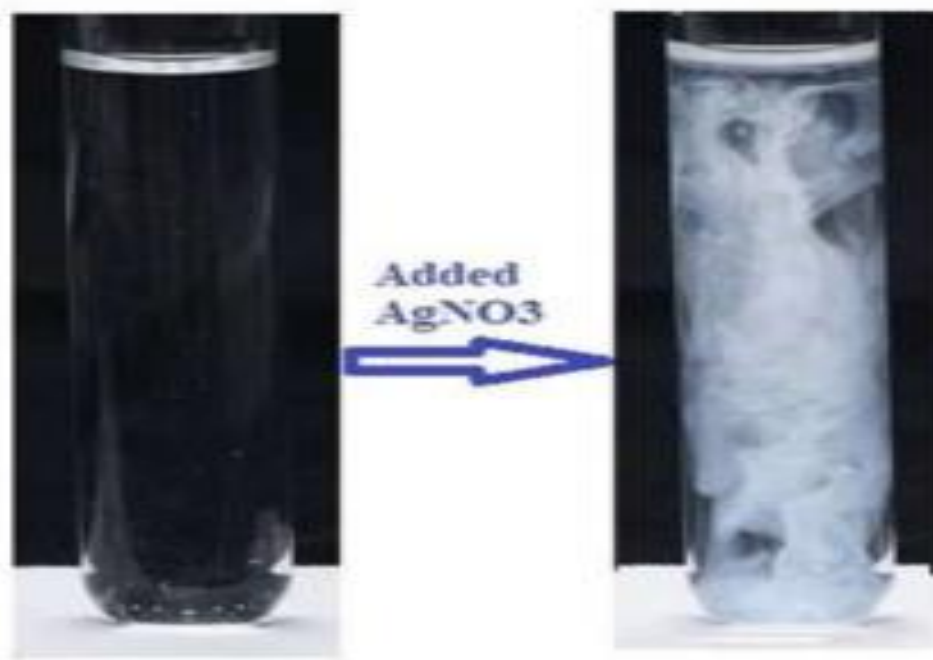
The silver chloride produced in the presence of dilute nitric acid makes the test solution turbid, the extent of turbidity/opalescence depending upon the amount of chloride present in the substance is compared with a standard turbidity/opalescence produced by addition of silver nitrate to a standard solution having a known amount of chloride and the same volume of dilute nitric acid as used in the test solution. If the turbidity/opalescence from the sample has been less than the standard solution, the sample will pass the limit test and vice versa. Dilute nitric acid is used in the limit test of chloride to make solution acidic and which helps silver chloride precipitate to make solution turbid at the end of process.

Chemical Reaction:



Procedure:

| Test sample | Standard compound (25 ppm Cl-) |
|--|---|
| Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder | Take 10 ml standard solution of chloride (25ppm Cl) in Nessler cylinder |
| Add 1.0 ml of nitric acid | Add 1.0 ml of nitric acid |
| Dilute to 50ml in Nessler cylinder | Dilute to 50ml in Nessler cylinder |
| Add 1ml of AgNO ₃ solution | Add 1ml 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 for chloride and vice-versa.



NOTE:

1. **Chloride standard solution (25 ppm Cl):** Dilute 5 mL of 0.0824% w/v solution of sodium chloride to 100 mL with distilled water.
2. **Dilute nitric acid:** Dilute 1.06 mL of conc. Nitric acid in sufficient distilled water to produce 100 mL.
3. **0.1 M Silver nitrate:** Dissolve 1.7 g of silver nitrate to 100 mL with distilled water.
4. Insoluble substances like magnesium trisilicate or light kaolin are first boiled with a mixture of water and dilute nitric acid. The solution is filtered and the filtrate is subjected to the test.
5. Coloured substances if present may be specially treated. For example, potassium permanganate is decolourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test. The reducing substances which would otherwise react with silver nitrate

in the chloride limit test are oxidized.

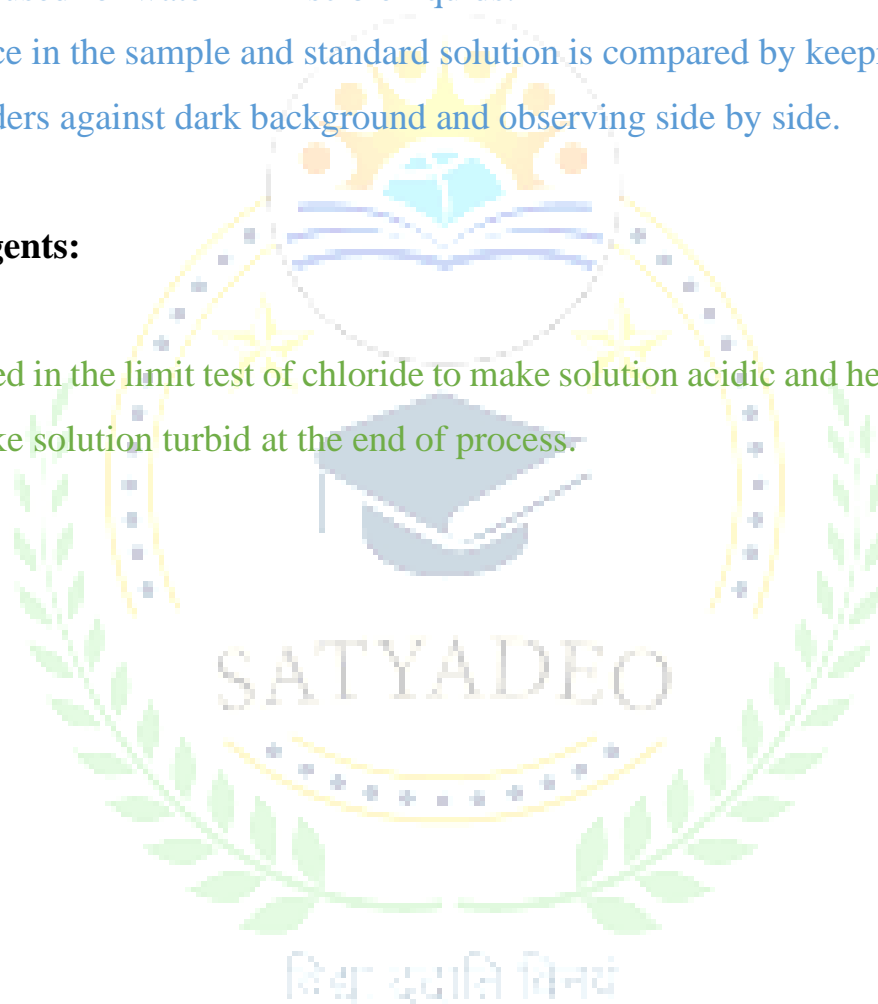
Reaction:



6. The quantitative and semi-quantitative determination of chloride contamination is also possible for water-soluble organic compounds.
7. This test is not used for water-immiscible liquids.
8. The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

Roles of the reagents:

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.

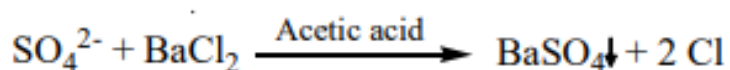
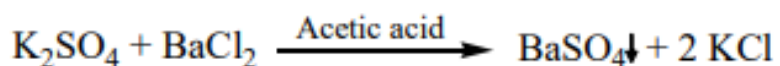


Limit Test for Sulphate

Principle:

- The limit test for sulphate is carried out on the basis of the reaction between barium chloride and soluble sulphates in the presence of Acetic acid.
- Then, the comparison of the turbidity produced by a given amount of the substance is done with standard turbidity obtained from a known amount of sulphates.
- The barium chloride 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. The ionic concentrations in the reagent has been so adjusted that the solubility product of barium sulphate gets exceeded, and the very small amount of barium sulphate present in the reagent acts as a seeding agent for precipitation of barium sulphate, if sulphate be present in the substance under test.
- Alcohol helps to prevent supersaturation and thus produces a more uniform opalescence/turbidity.
- Acetic acid helps to make solution acidic and barium sulphate precipitate formed is insoluble which gives turbidity/opalescence.

Reactions:



Procedure:

Take two 50 mL Nessler Cylinders. Label one as “Test” and the other as „Standard“.

| Test sample | Standard compound |
|---|--|
| Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transfer to a Nessler cylinder | Mix 15 mL of sulphate standard solution and 15 mL of distilled water in a Nessler cylinder |
| Add 0.15 mL of 5.0 M acetic acid | Add 0.15 mL of 5.0 M acetic acid |
| Add 2.5 mL of barium sulphate reagent | Add 2.5 mL of barium sulphate reagent |
| Add sufficient distilled water to produce 50 mL | Add sufficient distilled water to produce 50 mL |
| Stir immediately with glass rod and allow to stand for 5 minutes protected from light Keep aside for 5 min | |
| Observe the Turbidity | Observe the Turbidity |

Alternate 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 1ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder |
| Add 2ml of dilute hydrochloric acid | Add 2ml of dilute hydrochloric acid |
| Dilute to 45 ml in Nessler cylinder | Dilute to 45 ml in Nessler cylinder |
| Add 5ml of barium sulphate reagent | Add 5ml of barium sulphate reagent |
| Keep aside for 5 min | Keep aside for 5 min |
| Observe the Turbidity | Observe the 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 for sulphates and vice-versa.



Note:

- 1. Barium Sulphate Reagent:** Mix 10 mL of 25% w/v solution of barium chloride and 15 mL of ethanolic sulphate standard solution (10 ppm SO_4) and allow standing for 1 minute. It should always be prepared fresh.
- 2. Sulphate standard solution (10 ppm SO_4):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in distilled water to 100 mL with the same solvent.
- 3. Ethanolic sulphate standard solution (10 ppm SO_4):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 mL with the same solvent.
- 4. 5.0 M Acetic acid:** Dilute 28.5 mL of glacial acetic acid in sufficient distilled water to

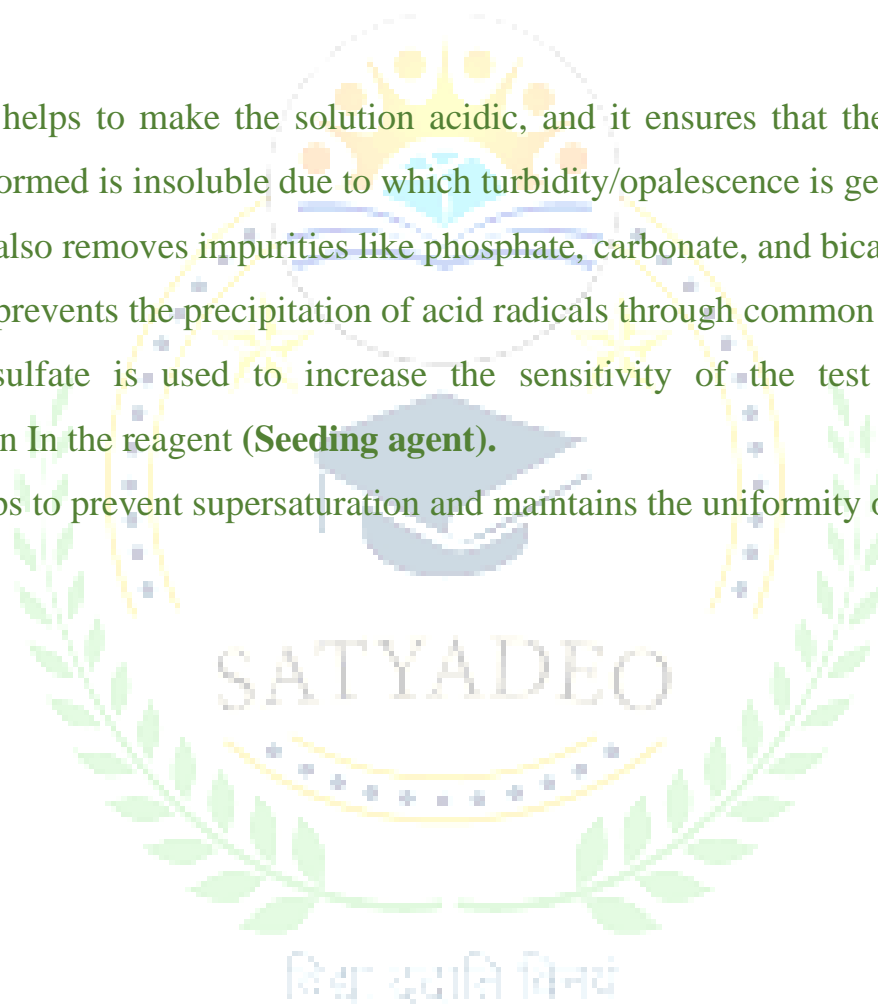
produce 100 mL.

5. This test is not used for water-immiscible liquids.

6. The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

Roles of reagents.

1. Acetic acid helps to make the solution acidic, and it ensures that the barium sulphate precipitate formed is insoluble due to which turbidity/opalescence is generated.
2. Acetic acid also removes impurities like phosphate, carbonate, and bicarbonate.
3. Acetic acid prevents the precipitation of acid radicals through common ion effect
4. Potassium sulfate is used to increase the sensitivity of the test by giving ionic concentration In the reagent (**Seeding agent**).
5. Alcohol helps to prevent supersaturation and maintains the uniformity of turbidity.



Limit Test for Iron

Principle:

It depends on the reaction of iron in an ammonical solution with thioglycollic acid in the presence of citric acid when a pale pink to deep reddish purple colour is produced.

The colour is due to the formation co-ordination compound, ferrous thioglycollate which is stable in the absence of air but fades in air due to oxidation.

Therefore, the colour should be compared immediately after the time allowed for full development of colour is over.

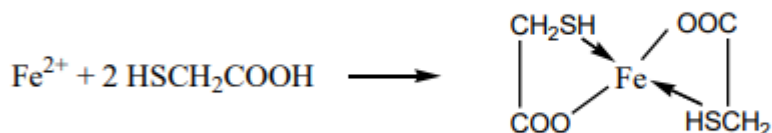
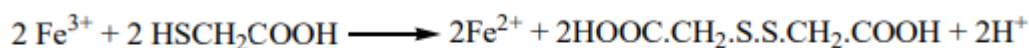
Ferrous thioglycollate is colourless in neutral or acid solutions. The colour develops only in the presence of alkali.

The original state of iron is immaterial, as thioglycollic acid reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) form.

Citric acid forms a soluble complex with iron and prevents its precipitation by ammonia as ferrous hydroxide.

Interference of other metal cations is eliminated by making use of citric acid, which forms complex with other metal cations.

Reactions:



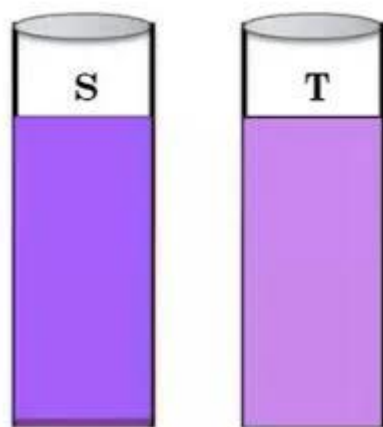
Ferrous Thioglycollate
Complex

Procedure:

Take two 50 mL Nessler Cylinders. Label one as “Test” and the other as „Standard“.

| Test sample | Standard compound (20 ppm Fe) |
|---|--|
| The sample is dissolved in a specific amount of water and then the volume is made up to 40 ml | 2 ml of the standard solution of iron diluted with water upto 40ml |
| 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 |

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.

**Note:**

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

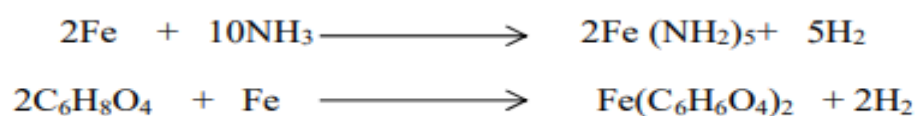
2. Standard Iron Solution: Weigh accurately 0.1726 g of ferric ammonium sulphate and

dissolve in 10 mL of 0.1 N sulphuric acid and sufficient distilled water to produce 1 Litre. Each mL of this solution contains 0.02 mg of Fe.

3. The colour developed in the sample and standard solution is compared by keeping the Nessler's cylinder against dark background and observing side by side

Roles of reagents.

1. Citric acid helps to prevent precipitation of iron by ammonia by forming a complex with it. It keeps iron in the solution form even in the presence of ammonia by forming a complex.



2. Citric acid (iron-free) is used to complex metal cations other than iron if any present.
3. Thioglycolic acid performs the following two functions:
 - I) Iron impurities may be present in the trivalent ferric form (Fe^{3+}) or in the divalent ferrous form (Fe^{2+}). If it is present in ferric form then thioglycolic acid reduces it to ferrous form.

$$\text{Fe}^{3+} + 2\text{HSCH}_2\text{COOH} \longrightarrow \text{Fe}^{2+} + 2\text{HSCH}_2\text{COO}^- + 2\text{H}^+$$
 - II) Thioglycolic acid produces purple color with the ferrous iron in the ammoniacal alkaline medium and in presence of citric acid.
4. Ammonia to make the solution alkaline

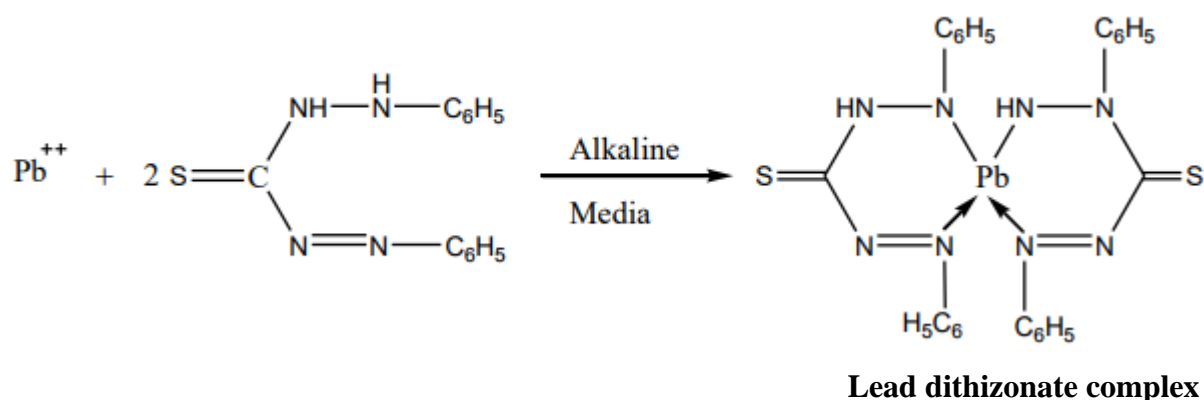
Limit Test for Lead

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 between lead and diphenyl thiocabazone (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.
- The intensity of the colour of complex is dependent upon the amount of lead in the solution. The colour of the lead-dithizone complex in chloroform has been compared with a standard lead solution, treated in the same manner.
- In this method, the lead present as an impurity in the substances, is separated by extracting an alkaline solution with a dithizone extraction solution. The interference and influence of other metal ion etc., is eliminated by adjusting the optimum pH for the extraction, by using ammonium citrate, potassium cyanide, hydroxylamine hydrochloride reagents, etc.

Reaction:-



Apparatus: (Separating Funnel)**Procedure:**

| Test sample | Standard compound |
|---|---|
| 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 |



Infographic Representation for lead test

Observation:

The intensity of the color of complex will depend on the amount of lead in the solution. The color produced in the 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.

Preparation of Reagents:

- 1% v/v nitric acid:** Dilute 1 vol of nitric acid to 100 volumes with water.
- Ammonium citrate solution Sp:** Dissolve 40 g of citric acid in 90 ml of water, add 2 drops of phenol red solution and then add slowly strong ammonia solution until the solution acquires a reddish colour. Remove any lead present by extracting the solution with successive quantities, each of 30 ml, of dithizone extraction solution until the dithizone solution retains its orange-green colour.
- Dithizone extraction solution:** Dissolve 30 mg of dithizone in 1000 ml of chloroform and add 5 ml of ethanol (95%). Store the solution in a

refrigerator. Before use, shake a suitable volume of the solution with about half its volume of a 1% v/v solution of nitric acid and discard the acid.

4. ***Dithizone standard solution:*** Dissolve 10 mg of dithizone in 1000 ml of chloroform. Store the solution in a glass-stoppered, lead-free, light-resistant bottle in a refrigerator.
5. ***Hydroxylamine hydrochloride solution Sp:*** Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to produce about 65 ml. Transfer to a separator, add 5 drops of thymol blue solution and strong ammonia solution until the solution becomes yellow. Add 10 ml of a 4 % w/v solution of sodium di-ethyl-di-thio carbamate and allow to stand for 5 minutes. Extract with successive quantities, each of 10 ml of chloroform until a 5 ml portion of the extract does not acquire a yellow colour when shaken with dilute cupric sulphate solution. Add dilute hydrochloric acid until the solution is pink and then with sufficient water to produce 100 ml.
6. ***Lead standard solution (0.1% Pb):*** Dissolve 0.400 g of lead nitrate in water containing 2 ml of nitric acid and add sufficient water to produce 250.0 ml.
7. ***Lead standard solution (1 ppm Pb):*** Dilute 1 volume of lead standard solution (10 ppm Pb) to 10 volumes with water.
8. ***Lead standard solution (10 ppm Pb):*** Dilute 1 volume of lead standard solution (100 ppm Pb) to 10 volumes with water.

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.

Roles of reagents:

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.



Limit Test for Arsenic

Arsenic is an undesirable and harmful type of impurity in pharmaceutical substances because it is toxic and cumulative in nature. The IP prescribes the limits for the presence of arsenic (NMT 2 ppm) as an impurity in various pharmaceutical substances [for example, NaCl should not contain more than 1 ppm]

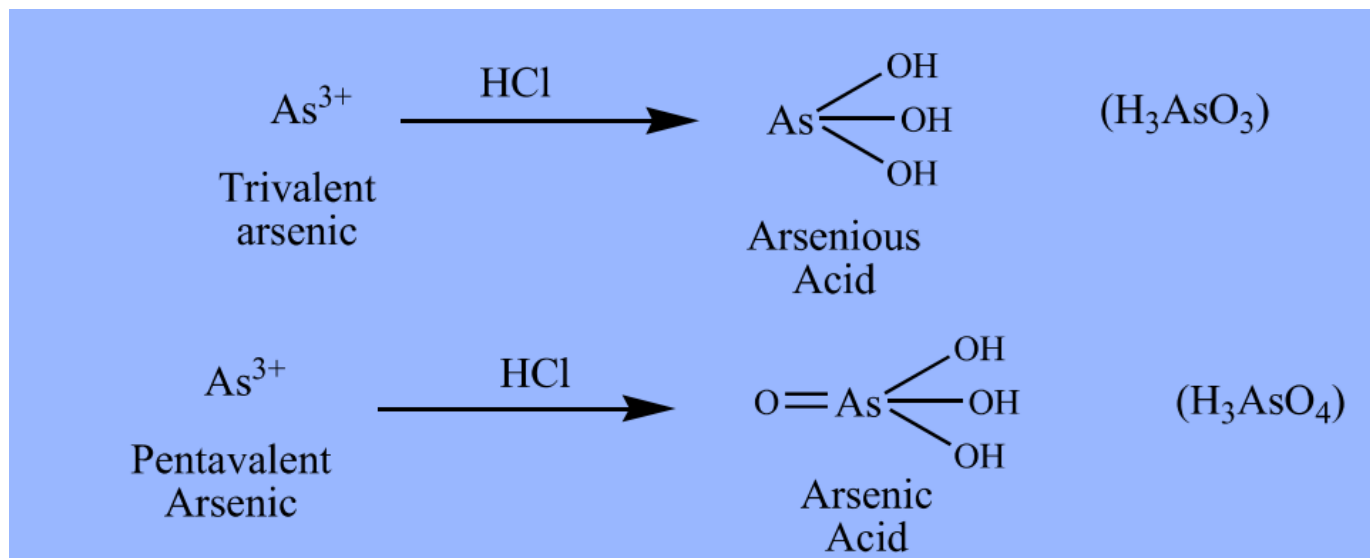
Principle:

Limit Test for Arsenic (As) is based on the fact that Arsenic is easily reduced into Arsine gas (AsH_3), which on mercuric chloride paper gives yellow stain.

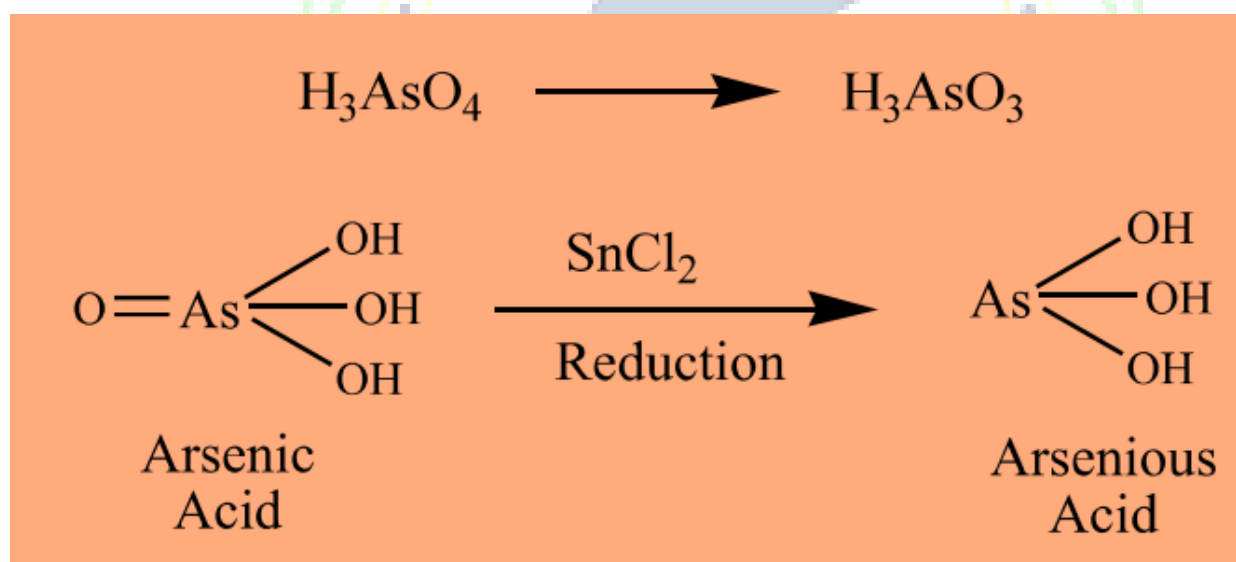
The principle is based on converting any arsenic impurity present in the sample to arsine gas by a series of reaction. The arsine gas is made to come in contact with mercuric chloride test paper whereby it produces a yellow or brown stain due to the formation of mercuric arsenic, depending upon the amount of impurity present in the given sample solution, then compared with arsenic standard solution containing 1ppm of arsenic ions, prepared by arsenic trioxide.

It is also called as **Gutzeit test** and requires special apparatus. Arsenic, present as arsenic acid in the sample is reduced to **arsenious acid** by reducing agents like **potassium iodide, stannous acid, zinc, hydrochloric acid**, etc. Arsenious acid is further reduced to **arsine (gas)** by hydrogen and **reacts with mercuric chloride paper to give a yellow stain**.

Arsenic (As) may be present as As^{3+} (Trivalent) or As^{5+} (Pentavalent)



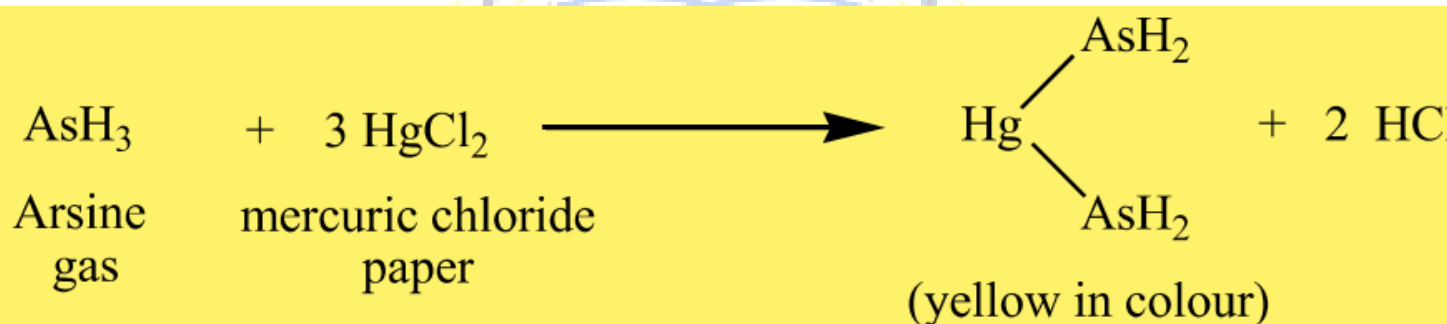
The solution is treated with a reducing agent (stannous Chloride) to convert the pentavalent arsenic acid into trivalent arsenious acid



The arsenious acid is then converted into gaseous arsenious hydride (arsine gas) with the help of nascent hydrogen, which is produced by $\text{Zn} + \text{HCl}$.



Arsine gas is carried through the tube by the stream of hydrogen and out through the mercuric chloride paper



This results in the formation of yellow or brown stain on the mercuric chloride paper.

The intensity of the colour is proportional to the quantity of arsenic.

In the same manner a standard stain is separately produced for the permissible limit of arsenic

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

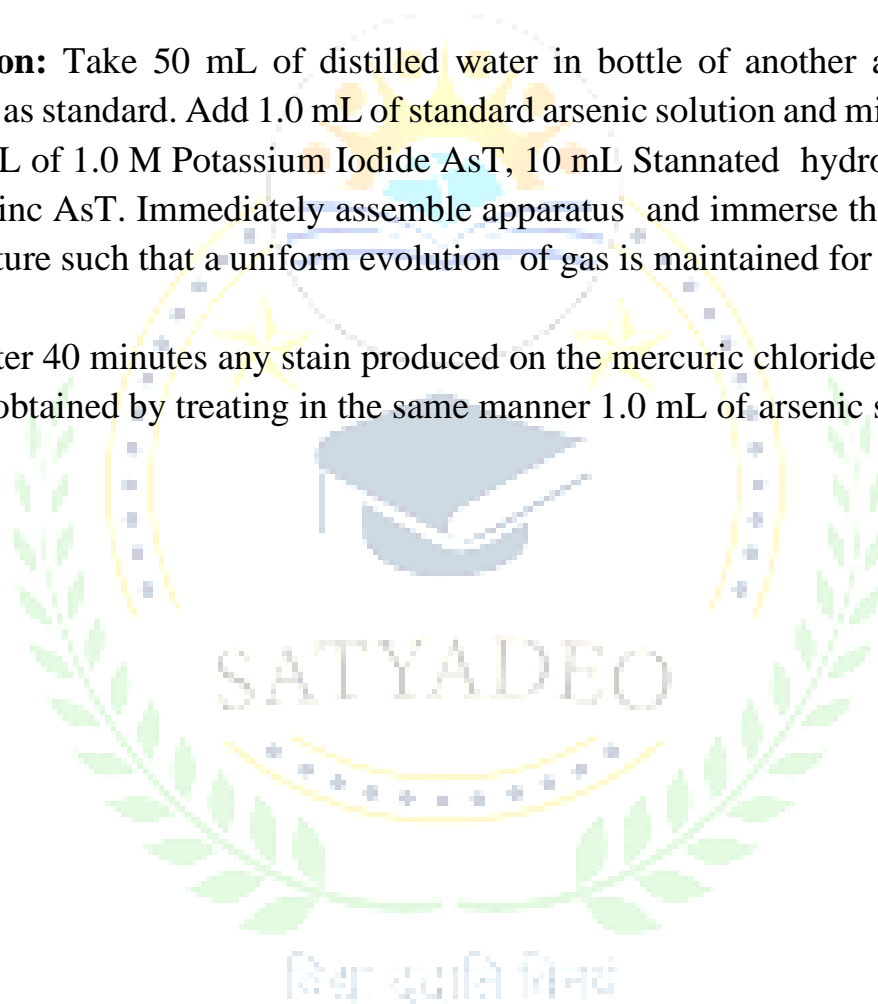
The intensity of the two stains is compared. If the intensity of stain in the case of 'Test' (sample) is more than that of the 'standard' then the sample contains more arsenic than the limit.

Procedure:

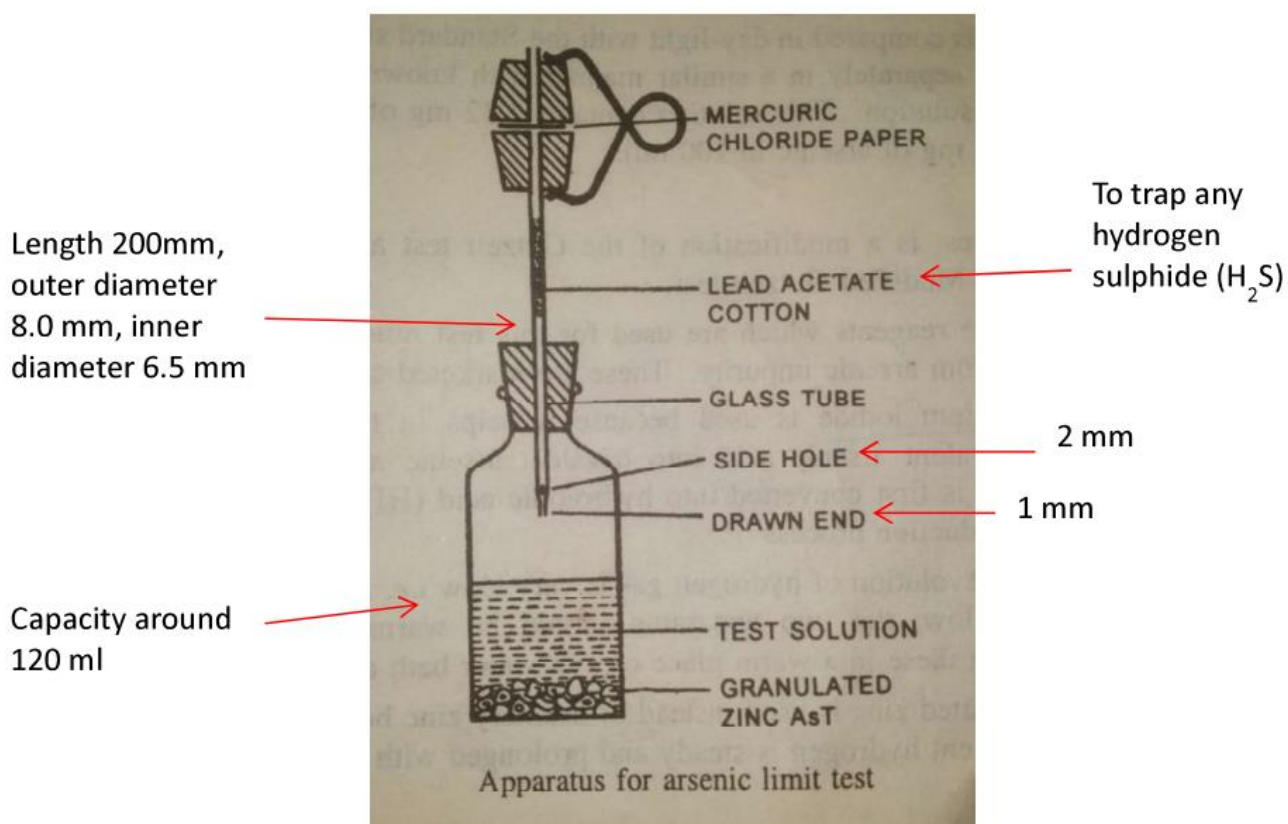
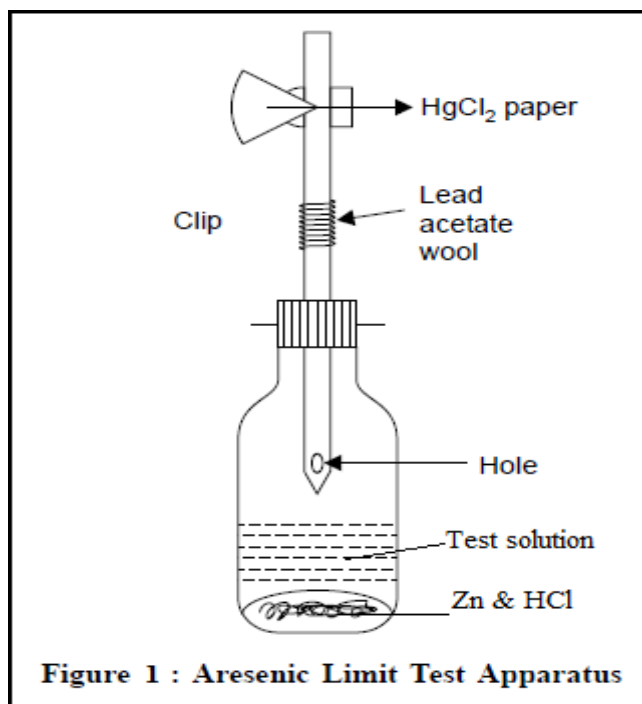
Test Solution: Dissolve 2.5 g of sample (ammonium chloride) in 50 mL of distilled water contain in the bottle of arsenic limit test apparatus labeled as test. To the above solution add 5 mL of 1.0 M Potassium Iodide AsT, 10 mL Stannated hydrochloric acid AsT and 10 g granulated zinc AsT. Immediately assemble apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained for 40 minutes.

Standard Solution: Take 50 mL of distilled water in bottle of another arsenic limit test apparatus labeled as standard. Add 1.0 mL of standard arsenic solution and mix it. To the above solution, add 5 mL of 1.0 M Potassium Iodide AsT, 10 mL Stannated hydrochloric acid AsT, 10 g granulated zinc AsT. Immediately assemble apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained for 40 minutes.

Observation: After 40 minutes any stain produced on the mercuric chloride paper is not more intense than that obtained by treating in the same manner 1.0 mL of arsenic standard solution.



Apparatus: (Gutzeit Apparatus or Arsenic test apparatus)



All reagent used must be completely free from arsenic impurity. Marked as 'AsT'.

Apparatus as per IP 1996:

1. This apparatus has following parts:
2. Approximately 60 ml generator bottle with 40 ml indicating line.
3. Glass tube with 6.5 mm inner diameter
4. A ground joint glass tube with 6.5 mm inner diameter and 18 mm outer diameter at the joint. Inner joint and the outer joint form a concentric circle.
5. Rubber stopper
6. Narrow part of the glass tube B. Glass wool is inserted up to this part.
7. Lead acetate cotton plug or wool

The apparatus (Fig) consists of a 100-ml bottle or conical flask closed with a rubber or ground glass stopper through which passes a glass tube (about 20 cm × 5 mm). The lower part of the tube is drawn to an internal diameter of 1.0 mm, and 15 mm from its tip is a lateral orifice 2 to 3 mm in diameter. When the tube is in position in the stopper the lateral orifice should be at least 3 mm below the lower surface of the stopper. The upper end of the tube has a perfectly flat surface at right angles to the axis of the tube. A second glass tube of the same internal diameter and 30 mm long, with a similar flat surface, is placed in contact with the first and is held in position by two spiral springs or clips. Into the lower tube insert 50 to 60 mg of lead acetate cotton, loosely packed, or a small plug of cotton and a rolled piece of lead acetate paper weighing 50 to 60 mg. Between the flat surfaces of the tubes place a disc or a small square of mercuric chloride paper large enough to cover the orifice of the tube (15 mm × 15 mm).

Notes:

Potassium iodide is used because it helps in the reduction of pentavalent arsenic acid into trivalent arsenic acid.

The tube must be washed with arsenic free hydrochloric acid, rinsed with water and dried between successive tests.

Carry out the test and the standard simultaneously using approximately similar time period.

Mercuric chloride paper should be protected from sunlight during the test to avoid lighter or no stain. If the stain present in the filter paper becomes dark, the test should be repeated by using pure reagents.

The reaction may be expedited by the application of heat and 40°C is considered to be the most ideal temperature.

Cotton wool dipped in lead acetate solution is used to trap any hydrogen sulphide gas liberated with arsine gas.

Connect tightly the ground joints holding the mercuric chloride test paper so that the gas produced does not leak out

Solution Preparation

1. **1M potassium iodide:** Dissolve 166.0 g of potassium iodide in sufficient water to produce 1000 ml.
2. **2 M sodium hydroxide:** Solution of any molarity xM may be prepared by dissolving 40x of sodium hydroxide in sufficient water to produce 1000 ml.
3. **Arsenic standard solution (10 ppm As):** Dissolve 0.330 g of arsenic trioxide in 5 ml of 2 M sodium hydroxide and dilute to 250.0 ml with water. Dilute 1 volume of this solution to 100 volumes with water.

Arsenic Standard Solution (1 ppm As): Dilute 1 volume of arsenic standard solution (10 ppm As) to 10 volumes with water.

4. **Lead acetate cotton:** Immerse absorbent cotton in a mixture of 10 volumes of lead acetate solution and 1 volume of 2 M acetic acid. Drain off the excess of liquid by placing it on several layers of filter paper without squeezing the cotton. Allow to dry at room temperature. Store in tightly-closed containers.
5. **Lead acetate paper:** Prepare from lead acetate solution and dry the impregnated paper at 100°C, avoiding contact with metal.
6. **Lead acetate solution:** A 10.0% w/v solution of lead acetate in carbon- di-oxide free water.
7. **Mercuric chloride paper:** Smooth white filter paper, not less than 25mm in width, soaked in a saturated solution of mercuric chloride, pressed to remove superfluous solution and dried at about 60° in the dark. The grade of filter paper is such that the weight is between 65 and 120 g per sq.m; the thickness in mm of 400 papers is approximately equal numerically, to the weight in g per sq.m.
8. **Stannated hydrochloric acid:** Stannated hydrochloric acid, low in arsenic, of commercial grade or prepared by adding 1 ml of stannous chloride solution AsT to 100 ml of hydrochloric acid AsT.
9. **Stannous chloride solution AsT:** Stannous chloride solution, low in arsenic, commercially available or prepared from stannous chloride solution by adding an equal volume of hydrochloric acid AsT, reducing to the original volume by boiling and filtering through a fine-grain filter paper.

Note: Use directly the sample in case of liquids.

Reasons:

1. Stannous chloride is used for complete evolution of arsine
2. Zinc, potassium iodide and stannous chloride is used as a reducing agent
3. HCl is used to make the solution acidic
4. Lead acetate pledger or papers are used to trap any hydrogen sulphide which may be evolved along with arsine.
5. Lead acetate cotton is used to remove traces of hydrogen sulphide (in the arsine and hydrogen gas) which is formed due to presence of any sulphide impurities.
6. The nascent hydrogen produced by the reaction of zinc and hydrochloric acid reduces arsenous acid to arsine gas.
7. Stannated hydrochloric acid is used for steady and uniform liberation of hydrogen gas from zinc. Since, zinc is not very reactive toward hydrochloric acid, tin forms Sn/Zn couple and makes reaction of zinc and hydrochloric acid faster.
8. Stannous chloride present in stannated hydrochloric acid reduces arsenic (As^{5+}) to arsenous (As^{3+}). Hydrogen gas liberated also act as a carrier gas for arsine.
9. A side hole at the lower end of the tube in arsenic apparatus prevents condensed liquid from being forced up the tube by the pressure of hydrogen, thus preventing blockade. Potassium iodide is also added to reduce arsenic to arsenous.

Limit Test for Heavy Metals

Introduction:

According to the International Union of Pure and Applied Chemistry or IUPAC, the term "heavy metal" may be a "meaningless term" because there is no standardized definition for a heavy metal. Some light metals or metalloids are toxic, while some high-density metals are not. For example, cadmium generally is considered a heavy metal, with an atomic number of 48 and specific gravity of 8.65, while gold typically is not toxic, even though it has an atomic number of 79 and specific gravity of 18.88. For a given metal, the toxicity varies widely depending on the allotrope or oxidation state of the metal. Hexavalent chromium is deadly; trivalent chromium is nutritionally significant in many organisms, including humans.

What are heavy metals?

A heavy metal is a metallic element which is toxic and has a high density, specific gravity or atomic weight. For examples: lead, mercury, cadmium, cobalt, chromium, lithium etc

Source: Tap water/ ground water/mineral water/ chemical reagents containing heavy metals, containers made up of heavy metals

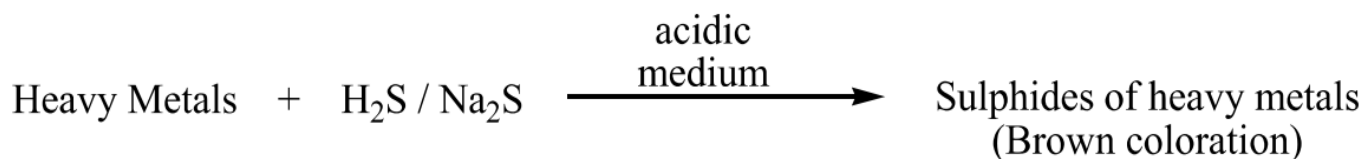
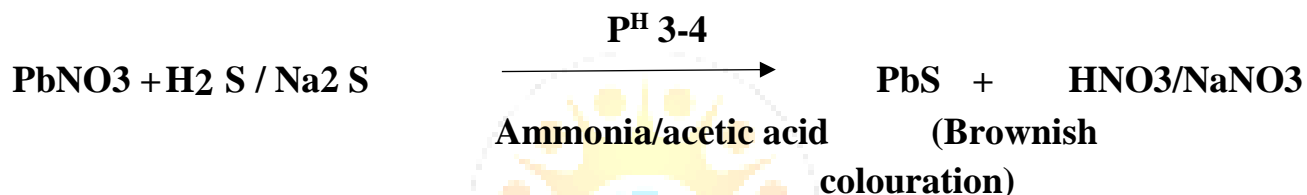
Limit test of heavy metals:

Metals that 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

Principle:

Limit test of heavy metals is based upon the chemical reaction between metallic impurities with freshly prepared saturated solution of hydrogen sulphide at Ph 3-4 to form brownish colloidal colour solution of respective metallic sulphides. The intensity of colour developed depends upon the amount of impurity present within the sample solution which is then compared with a standard solution prepared similarly by using lead nitrate containing 20 PPM of lead ions.

If the colour intensity produced in the sample is less than the standard, it passes the limit test. If it is more than the standard, it fails the limit test, when performed and viewed vertically downwards in a matched Nessler's cylinder under a white back ground.

Chemical reactions:**Standard solution:**

Here hydrogen sulphide gas is generated using a specially devised apparatus called as Kipp's apparatus in which ferrous sulphide sticks are made to react with equal volumes of concentrated hydrochloric acid and water.

Indian pharmacopoeia 1996, provides four methods depending on the resulting solution of substance (i.e., based on solubility, colour etc). Method A uses hydrogen sulphide solution, method B uses hydrogen sulphide solution after igniting the substance, method C uses sodium sulphide solution after treating the substance with sodium hydroxide solution, and in method D thioacetamide solution is used. In a concise way, the methods can be categorized as follows:

Method A & B: Method A is used for substance gives clear colorless solution under specific condition given in monograph, while Method B is for those substance which does not give clear colorless solution.

Method C & D: Method C is used for the substances which gives clear colourless solution in sodium hydroxide, while Method D is used for substance which does not gives clear colourless solution in NaOH solution.

Procedure:

The Indian Pharmacopoeia has adopted four methods for the limit test of heavy metals.

Method A:

Use for the substance which gives clear colorless solution under the specific condition.

| Test sample | Standard compound |
|--|--|
| Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder | Take 2 ml of standard lead solution and dilute to 25 ml with water |
| 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 |
| Dilute with water to 35 ml | Dilute with water to 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 five minutes | Allow to stand for five minutes |
| View downwards over a white surface | View downwards over a white surface |

Observation:

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 heavy metals and vice versa.

Method B:

| Test sample | Standard compound |
|--|--|
| Weigh specific quantity of test substance, moisten with sulphuric acid and ignite on a low flame till completely charred Add few drops of nitric acid and heat to 500 °C Allow to cool and add 4 ml of hydrochloric acid and evaporate to dryness Moisten the residue with 10 ml of hydrochloric acid and digest for two minutes Neutralize with ammonia | Take 2 ml of standard lead solution and dilute to 25 ml with water |

| | |
|--|--|
| solution and make just acid with acetic acid | |
| Adjust the pH between 3 to 4 and filter if necessary | Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution |
| Dilute with water to 35 ml | Dilute with water to 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 five minutes | Allow to stand for five minutes |
| View downwards over a white surface | View downwards over a white surface |

Use for the substance which do not give clear colorless solution under the specific condition.

Observation:

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 heavy metals and vice versa.

Method C:

Use for the substance which gives clear colorless solution in sodium hydroxide solution.

| Test sample | Standard compound |
|--|---|
| 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 sodium hydroxide solution | Take 2 ml of standard lead solution |
| Make up the volume to 50 ml with water | Add 5 ml of dilute sodium hydroxide solution and 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 |

Observation:

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 heavy metals and vice versa.

Method D:**Standard Solution:**

Pipette 10.0 ml of either standard lead solution (1 ppm Pb) or standard lead solution (2 ppm Pb) into a small Nessler cylinder labeled as "Standard". Add 2.0 ml of the test solution and mix.

Test Solution:

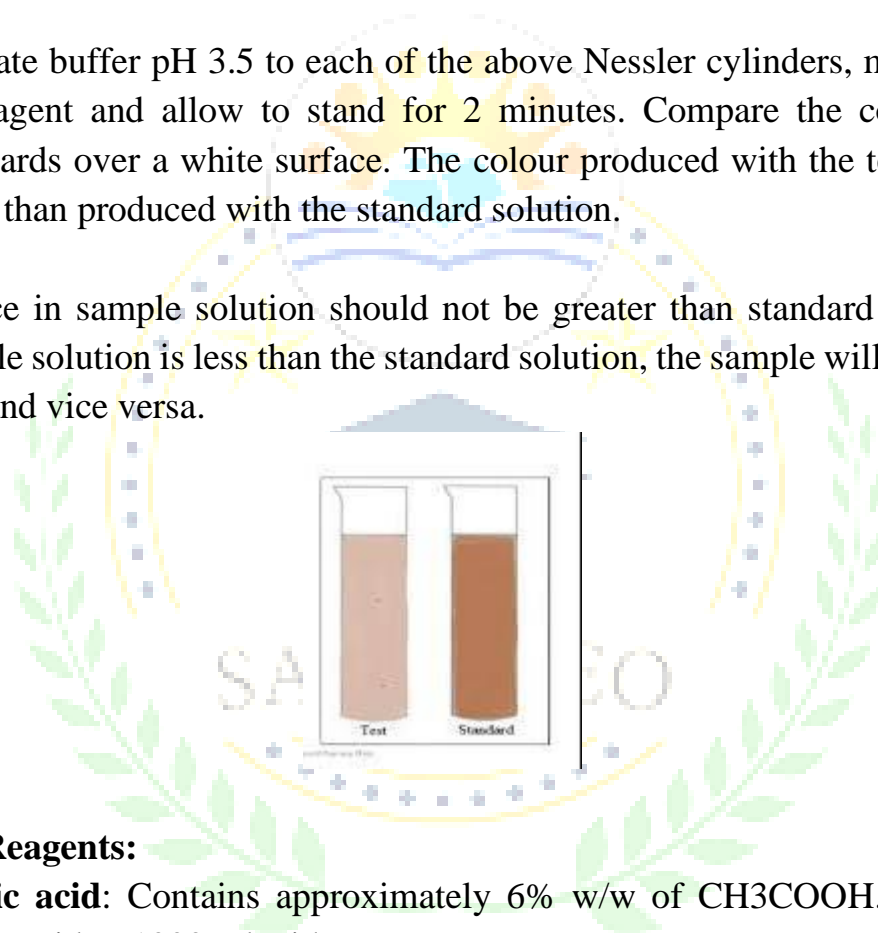
Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder labeled as "Test".

Procedure:

Add 2 ml of acetate buffer pH 3.5 to each of the above Nessler cylinders, mix, add 1.2 ml of thioacetamide reagent and allow to stand for 2 minutes. Compare the colour by viewing vertically downwards over a white surface. The colour produced with the test solution is not more intense than that produced with the standard solution.

Observation:

The color produced in sample solution should not be greater than standard solution. If color produced in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

**Preparation of Reagents:**

1. **Dilute acetic acid:** Contains approximately 6% w/w of CH_3COOH . Dilute 57 ml of glacial acetic acid to 1000 ml with water.
2. **Dilute ammonia solution:** Contains approximately 10% w/w of NH_3 . Dilute 425 ml of strong ammonia solution to 1000 ml. Store in well-closed containers in a cool place.
3. **Lead Standard solution (0.1% Pb):** Dissolve 0.400 g of lead nitrate in water containing 2 ml of nitric acid and add sufficient water to produce 250.0 ml.
4. **Lead Standard solution (100 ppm Pb):** Dilute 1 volume of lead standard solution (0.1% Pb) to 10 volumes with water.
5. **Lead Standard solution (20 ppm Pb):** Dilute 1 volume of lead standard solution (100 ppm Pb) to 5 volumes with water.

6. **Dilute Sodium Hydroxide:** A 5.0% w/v solution of sodium hydroxide.

Role of reagents:

1. Dilute acetic acid or ammonia solution are used to maintain a pH of 3-4 as that at this particular pH the metallic impurities get converted into their respective metallic impurities.
2. Freshly prepared saturated solution of hydrogen sulphide: acts as a reagent for identifying the metallic impurities.
3. Dilute Sodium Hydroxide is used to provide alkaline reaction medium.



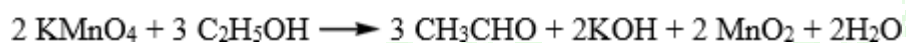
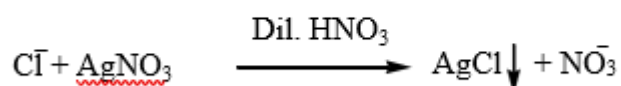
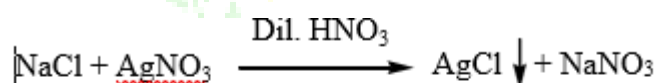
MODIFIED LIMIT TEST FOR CHLORIDES

Principle:

Modified limit test is performed; if the limit tests for a sample (Coloured compound) cannot be done by normal method. For example, potassium permanganate is de-colourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test.

It is based upon the chemical reaction between silver nitrate and soluble chlorides to obtain silver chloride in presence of dilute nitric acid. The silver chloride produced in the presence of dilute nitric acid makes the test solution turbid, the extent of turbidity/opalescence depending upon the amount of chloride present in the substance is compared with a standard turbidity/opalescence produced by 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. If the turbidity/opalescence from the sample has been less than the standard solution, the sample will pass the limit test and vice versa. Dilute nitric acid is used in the limit test of chloride to make solution acidic and which helps silver chloride precipitate to make solution turbid at the end of process.

Reactions:



Procedure:

Preparation of Test Solution (KMnO₄):

Dissolve 1.5 g in 50 mL of distilled water, heat on a water-bath and add gradually 6 mL of ethanol (95%), cool, dilute to 60 mL with distilled water and filter.

Take two 50 mL Nessler Cylinders. Label one as "Test" and the other as „Standard'.

| Test | Standard |
|---|---|
| Take 40 mL of the above test solution in Nessler cylinder. | Take 10 mL of chloride standard solution (250 ppm Cl) and 5 mL of water |
| Add 1.0 mL of dilute nitric acid | Add 1.0 mL of dilute nitric acid |
| Dilute to 50 mL with distilled water | Dilute to 50 mL with distilled water |
| Add 1.0 mL of 0.1 M <u>AgNO₃</u> solution | Add 1.0 mL of 0.1 M <u>AgNO₃</u> solution |
| Stir immediately with glass rod and allow to stand for 5 minutes protected from light | |

Observation: The opalescence produced in sample solution should not be greater than standard solution. If opalescence produced in sample solution is less than the standard solution, the sample will pass the limit test for chloride and vice-versa.

NOTE:

- 1. Chloride standard solution (250 ppm Cl):** Dilute 50 mL of 0.0824% w/v solution of sodium chloride to 100 mL with distilled water.
- 2. Dilute nitric acid:** Dilute 1.06 mL of conc. Nitric acid in sufficient distilled water to produce 100 mL.
- 3. 0.1 M Silver nitrate:** Dissolve 1.7 g of silver nitrate to 100 mL with distilled water.
- The quantitative and semi-quantitative determination of chloride contamination is also possible for water-soluble organic compounds.
- This test is not used for water-immiscible liquids.

The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

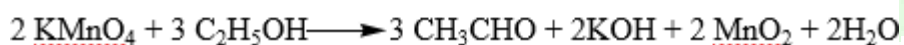
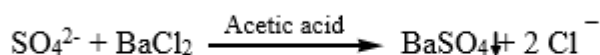
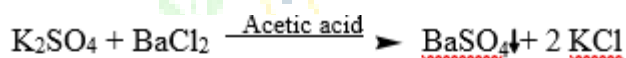
MODIFIED LIMIT TEST FOR SULPHATES

Principle:

Modified limit test is performed; if the limit tests for a sample (Coloured compound) cannot be done by normal method. For example, potassium permanganate is de-colourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test.

The limit test for sulphate is carried out on the basis of the reaction between barium chloride and soluble sulphates in the presence of acetic acid. Then, the comparison of the turbidity produced by a given amount of the substance is done with a standard turbidity obtained from a known amount of sulphate. The barium chloride 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. The ionic concentrations in the reagent has been so adjusted that the solubility product of barium sulphate gets exceeded, and the very small amount of barium sulphate present in the reagent acts as a seeding agent for precipitation of barium sulphate, if sulphate be present in the substance under test. Alcohol helps to prevent super-saturation and thus produces a more uniform opalescence/turbidity. Acetic acid helps to make solution acidic and barium sulphate precipitate formed is insoluble which gives turbidity/opalescence.

Reactions:



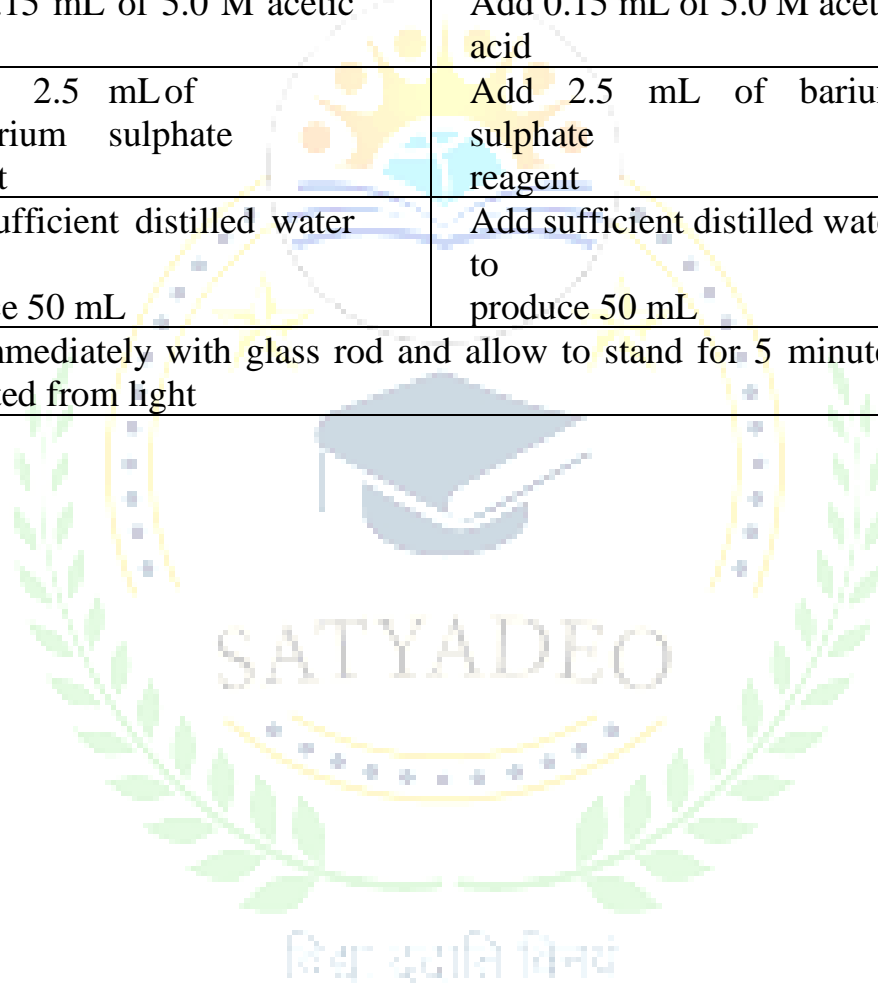
PROCEDURE

Preparation of Test Solution (KMnO₄):

Dissolve 1.5 g in 50 mL of distilled water, heat on a water-bath and add gradually 6 mL of ethanol (95%), cool, dilute to 60 mL with distilled water and filter.

Take two 50 mL Nessler Cylinders. Label one as "Test" and the other as „Standard’.

| Test | Standard |
|---|--|
| Take 10 mL of the above test solution in Nessler cylinder. | Mix 15 mL of sulphate standard solution and 15 mL of distilled water in a Nessler cylinder |
| Add 0.15 mL of 5.0 M acetic acid | Add 0.15 mL of 5.0 M acetic acid |
| Add 2.5 mL of barium sulphate reagent | Add 2.5 mL of barium sulphate reagent |
| Add sufficient distilled water to produce 50 mL | Add sufficient distilled water to produce 50 mL |
| Stir immediately with glass rod and allow to stand for 5 minutes protected from light | |



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 for sulphates and vice-versa.

Note:

- **Barium Sulphate Reagent:** Mix 10 mL of 25% w/v solution of barium chloride and 15 mL of ethanolic sulphate standard solution (10 ppm SO_4) and allow standing for 1 minute. It should always be prepared fresh.
- **Sulphate standard solution (600 ppm SO_4):** Dilute 60 mL of a 0.181% w/v solution of potassium sulphate in distilled water to 100 mL with the same solvent.
- **Ethanolic sulphate standard solution (10 ppm SO_4):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 mL with the same solvent.
- **5.0 M Acetic acid:** Dilute 28.5 mL of glacial acetic acid in sufficient distilled water to produce 100 mL.
- This test is not used for water-immiscible liquids.
- The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

