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Limit Tests

Introduction

Impurity is defined as the presence of one substance in another substance in low concentration. Impurity can be an organic, in-organic, microbial, dust, moisture etc. In a pharmaceutical substance, the nature of impurity can be predicted provided we know the source through which it has been obtained. Impurities are imparted into the pharmaceutical substance through raw materials, intermediates, reagents, catalysts, solvents, reaction vessels, improper storage, cross-contamination, manufacturing errors, packing errors, microbial contamination, chemical instability, storage containers etc.

Water is a rich source for chlorides, sulphates, carbonates etc. Reactor materials used for manufacturing are rich source of steel, copper, iron, zinc, lead. Reagents, catalysts are rich sources of arsenic, antimony, heavy metals, lead, cadmium, mercury, which are potent nerve poisons on cumulative accumulation.

Hence presence of an impurity in a pharmaceutical substance may cause cumulative toxic effect, decreased therapeutic effect, change in physical and chemical properties, difficulty in formulation, in-compatibility, decrease in shelf-life, change in odour, colour, taste and appearance.

Since procuring pure pharmaceutical substance free from impurities is expensive and difficult process, Indian Pharmacopoeia, which is under the control of Ministry of Health & Family Welfare, Government of India provides permissible limit of a impurity and designate the pharmaceutical substance as standard provided it complies the tests given under individual monographs.

Limit tests are quantitative or semi-quantitative test designed to control small quantities of in-organic impurities, which are likely to be present in a pharmaceutical substance. Limit test for chlorides, sulphates, lead, iron, heavy metals and arsenic are official tests designed and mentioned in the individual monograph of pharmaceutical substance in Indian Pharmacopoeia.

The limit of impurity is provided in terms of parts per million (ppm ≡ 1 μg ≡ 10^{-6}g).

In these tests, standard opalescence/turbidity/colour/stain obtained by the reaction of known quantity of impurity with the reagent is compared with the test opalescence/turbidity/colour/stain obtained by the reaction of specified quantity of test sample (pharmaceutical substance) with the reagent. Hence limit tests are comparative tests in which both test and standard must be prepared simultaneously at the same conditions. The reagents used are dilute solutions so that the reaction is slow and sensitive. Additionally, the reagent selected should be less specific so that limits of several likely impurities can be accomplished.

In case of limit test for chlorides, sulphates, heavy metals and iron, Nessler cylinders are used for the test and the standard. Nessler cylinders are matched tubes of clear, colourless glass with a uniform internal diameter and a flat, transparent base. They are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50 ml mark, 110 to 124 mm, the thickness of the wall, 1.0 to 1.5 mm and the thickness of the base, 1.5 to 3.0 mm. The external height to the 50 ml mark of the cylinders used for a test must not vary by more than 1mm.

***Note*:**

(i) Special alternations should be done while performing limit tests especially in case of insoluble and coloured pharmaceutical substances. In case of insoluble pharmaceutical substances (ex: activated charcoal), it is thoroughly extracted with boiling water and later the water is used for performing limit tests. In case of coloured pharmaceutical substances (ex: potassium permanganate), the colour is removed by chemical treatment like ethyl alcohol and later limit test is performed. One has to also remember that limit of impurity is fixed for individual pharmaceutical substance, but quantity of pharmaceutical substance to be tested for limit test is mentioned in individual monograph.

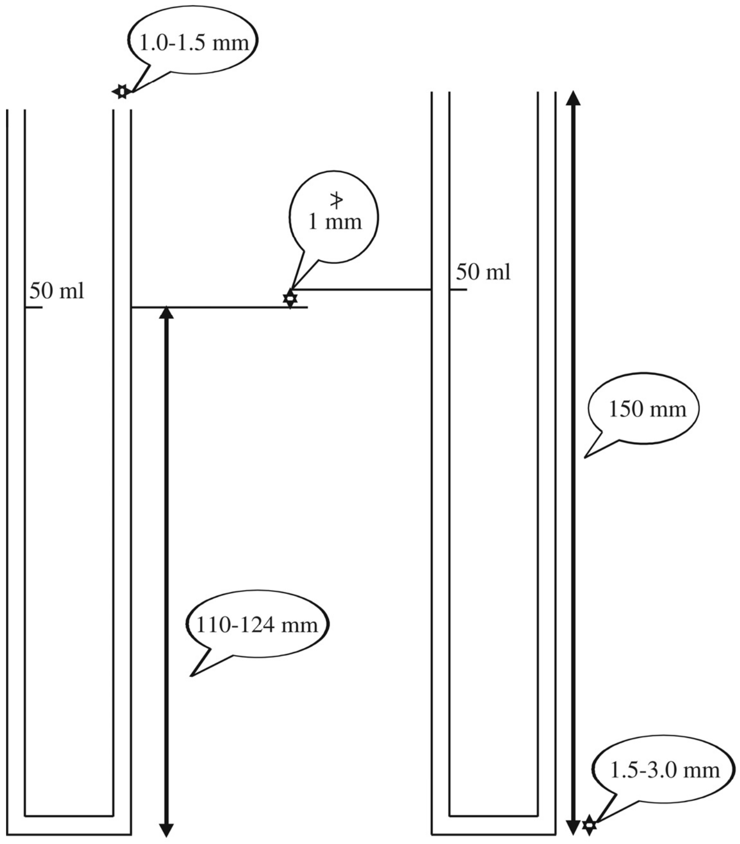
(ii) Use directly the sample in case of liquid or in solution form as directed in monographs.

(iii) If a test sample has to be made to fail in limit test for a particular impurity, add small quantity of standard impurity into the sample and perform the test.

(iv) A turbid solution is a non-clear solution. Opalescence is a change in colour.

(v) Select similar identical Nessler cylinders by keeping next to each other and the difference between 50 ml mark of both cylinders should not be more than 1 mm.

Diagram of Nessler Cylinders



Experiment 01

Limit Test for Chlorides

Aim: To perform limit test for chlorides for the given sample.

Requirements: Nessler cylinders, glass rod, measuring cylinders, 1ml bulb pipette, 10 ml bulb pipette, dilute nitric acid, 0.1 M silver nitrate, chloride standard solution (25 ppm Cl), test sample.

Principle: In this experiment, the test opalescence obtained by the reaction of chloride impurities with silver nitrate is compared with standard opalescence obtained by the reaction of known quantity of chloride with silver nitrate. Dilute nitric acid is used to dissolve other impurities if present.

$$Cl^- + AgNO_3 \xrightarrow{\text{Dil . }HNO_3} \underset{\text{opalescence}}{AgCl\downarrow} + NO_3^-$$

The precipitate silver chloride formed is insoluble in dilute nitric acid and gives opalescence.

Procedure:

Test opalescence: Dissolve the given sample in 20 ml of water and transfer to a Nessler cylinder. Add 10 ml of dilute nitric acid, dilute to 50 ml with water. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes, protected from light. View transversely against a black background.

Standard opalescence: Transfer 10.0 ml of chloride standard solution (25 ppm Cl) into a Nessler cylinder and add 5 ml of water. Add 10 ml of dilute nitric acid, dilute to 50 ml with water. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes, protected from light. View transversely against a black background.

Test Solution	Standard Solution
Dissolve the given sample in 20 ml of water and transfer to a Nesseler's cylinder.	Transfer 10.0 ml of standard chloride solution (25 ppm) in to a Nesseler's cylinder and add 5 ml water.
Add 10 ml of dilute nitric acid, dilute to 50 ml with water.	Add 10 ml of dilute nitric acid, dilute to 50 ml with water.
Add 1 ml of 0.1 M silver nitrate solution.	Add 1 ml of 0.1 M silver nitrate solution.
Stir immediately with a glass rod and allowed to stand for 5 min, protected from light and viewed transversely against a black background.	Stir immediately with a glass rod and allowed to stand for 5 min, protected from light and viewed transversely against a black background.

Observation:

Test opalescence is not more intense than standard opalescence.

or

Test opalescence is more intense than standard opalescence.

Report/Result:

The given sample passes limit test for chlorides.

or

The given sample fails limit test for chlorides.

Preparation of Reagents:

1. *0.1 M silver nitrate:* Dissolve 17.0 g of silver Nitrate in sufficient water to 1000 ml. (Store in light-resistant containers)
2. *Chloride standard solution (25 ppm Cl):* Dilute 5 volumes of a 0.0824% w/v of sodium chloride to 100 volumes with water.
3. *Dilute nitric acid:* Contains approximately 10% w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Experiment 02

Limit Test for Sulphates

Aim: To perform limit test for sulphates for the given sample.

Requirements: Nessler cylinders, glass rod, 1ml bulb pipette, 2 ml graduate pipette, 5 M acetic acid, sulphate standard solution (10 ppm SO_4), test sample, barium chloride solution, ethanolic sulphate standard solution (10 ppm SO_4), 1ml graduate pipette.

Principle: In this experiment, the test opalescence/turbidity obtained by the reaction of sulphate impurities with barium chloride is compared with standard opalescence/turbidity obtained by the reaction of known quantity of sulphate with barium chloride.

Dilute acetic acid is used to dissolve other impurities if present.

$$SO_4^{2-} + BaCl_2 \xrightarrow{\text{Dil. acetic acid}} \underset{\text{opalescence/turbidity}}{BaSO_4\downarrow} + 2Cl^-$$

The precipitate barium sulphate formed is insoluble in dilute acetic acid and gives opalescence/turbidity. A known amount of potassium sulphate is added both in test and standard in order to increase sensitivity, rapid and complete precipitation by seeding. Ethyl alcohol is used to prevent super-saturation and thus producing uniform opalescence/turbidity. In the earlier editions of Indian pharmacopoeia, barium sulphate reagent containing barium sulphate and ethyl alcohol was used instead of ethanolic sulphate standard solution. Instead of acetic acid, hydrochloric acid was used. The change in the reagent does not effect the objective of the test.

Procedure:

Test opalescence: To 1.0 ml of a 25.0% w/v solution of barium chloride in a Nessler cylinder add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute. Dissolve the given sample in 15 ml of water and add 0.15 ml of 5M acetic acid, pour the solution in the Nessler cylinder. Add sufficient water to produce 50 ml, stir immediately with a glass rod and allow to stand for 5 minutes and viewed transversely against a black back ground.

Standard opalescence: To 1.0 ml of a 25.0% w/v solution of barium chloride in a Nessler cylinder add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute. Add 15 ml of sulphate standard solution (10 ppm SO_4) and 0.15 ml of 5 M acetic acid. Add sufficient water to produce 50 ml, stir immediately with a glass rod and allow to stand for 5 minutes and viewed transversely against a black back ground.

Test Solution	Standard Solution
Transfer 1.0 ml of a 25 % w/v solution of barium chloride in to Nessler's cylinder.	Transfer 1.0 ml of a 25 % w/v solution of barium chloride in to Nessler's cylinder.
Add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix and allowed to stand for 1 min.	Add 1.5 ml of ethanolic sulphate standard solution (10ppm SO_4), mix and allowed to stand for 1 min.
Dissolve the given sample in 15 ml of water and add 0.15 ml of 5 M acetic acid and pour the solution into a Nessler's cylinder.	Add 15 ml of standard sulphate solution (10 ppm SO_4) and 0.15 ml of 5 M acetic acid to the Nessler's cylinder.
Add sufficient water to make up 50ml.	Add sufficient water to make up 50ml.
Stir immediately with a glass rod and allowed to stand for 5 min and viewed transversely against a black background.	Stir immediately with a glass rod and allowed to stand for 5 min and viewed transversely against a black background.

Observation:

Test opalescence is not more intense than standard opalescence.

or

Test opalescence is more intense than standard opalescence.

Report/Result:

The given sample passes limit test for sulphates.

or

The given sample fails limit test for sulphates.

Preparation of Reagents:

1. *25% w/v barium chloride:* Dissolve 25.0 g of barium chloride in 100 ml water.
2. *5 M acetic acid:* Solution of any molarity xM may be prepared by diluting $57x$ ml ($60x$ g) of glacial acetic acid to 1000 ml with water.

3. *Ethanolic sulphate standard solution (10 ppm SO_4):* Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 volumes with ethanol (30%).
4. *Sulphate standard solution (10 ppm SO_4):* Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in distilled water to 100 volumes with the same solvent.

Experiment 03

Limit Test for Iron

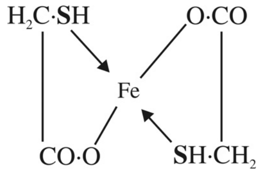
Aim: To perform limit test for iron for the given sample.

Requirements: Nessler cylinder, 2ml bulb pipette, 20% w/v iron-free citric acid, thioglycollic acid, ammonia solution, iron standard solution (20 ppm Fe), glass rods, red-litmus paper.

Principle: In this experiment, the test colour (purple) obtained by the reaction of iron impurities with mercaptoacetic acid (thio glycollic acid) is compared with standard colour obtained by the reaction of known quantity of iron with mercaptoacetic acid.

Citric acid (iron-free) is used to complex metal cations other than iron if any present.

$$2\,Fe^{3+} + 2\,SH{\bullet}CH_2{\bullet}COOH \longrightarrow 2\,Fe^{2+} + HOOC{\bullet}CH_2{\bullet}S{\bullet}S{\bullet}CH_2{\bullet}COOH + 2\,H^+$$

$Fe^{2+} + 2\,SH{\bullet}CH_2{\bullet}COOH \longrightarrow$  $+ 2\,H^+$

Ferrous Mercapto acetate/
Ferrous thioglycolate

In addition to forming complex with Fe^{2+}, thioglycollic acid acts as a reducing agent and converts Fe^{3+} to Fe^{2+} if any present.

The ferrous mercapto acetate formed gives purple colour in presence of citric acid. In addition to formation of metal complex other than iron, citric acid forms ammonium citrate buffer when ammonia is added to make alkaline, which in turn stablises the complex formed.

Procedure:

Test colour: Dissolve the given sample in 20 ml of water and transfer to a Nessler cylinder. Add 2 ml of a 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycollic acid, mix, make alkaline with iron-free ammonia solution, dilute to 50 ml with water and allow to stand for 5 minutes and observe the colour transversely.

Standard colour: Transfer 2.0 ml of iron standard solution (20 ppm Fe) to a Nessler cylinder. Dilute with 20 ml water. Add 2 ml of a 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycollic acid, mix, make alkaline with iron-free ammonia solution, dilute to 50 ml with water and allow to stand for 5 minutes and observe the colour transversely.

Test solution	Standard solution
Dissolve the given sample in 20 ml water and transfer into Nessler's cylinder.	Transfer 2.0 ml of iron standard solution (20 ppm Fe) to a Nessler's cylinder.
Add 2 ml of 20 % w/v of iron free citric acid.	Add 2 ml of 20 % w/v of iron free citric acid.
Add 0.1 ml of thioglycollic acid.	Add 0.1 ml of thioglycollic acid.
Then make the solution to alkaline with iron free ammonia solution.	Then make the solution to alkaline with iron free ammonia solution.
Dilute to 50 ml with water and allowed to stand for 5 min and observe the color transversely.	Dilute to 50 ml with water and allowed to stand for 5 min and observe the color transversely.

Observation:

Test colour is not more intense than standard colour.

or

Test colour is more intense than standard colour.

Report/Result:

The given sample passes limit test for iron.

or

The given sample fails limit test for iron.

Preparation of Reagents:

1. *0.05 M sulphuric acid:* Solutions of any molarity *x*M may be prepared by carefully adding 54*x* ml of sulphuric acid to an equal volume of water and diluting to 1000 ml with water.
2. *20% w/v iron-free citric acid:* Dissolve 20 g of iron-free citric acid in 100 ml water.
3. *Iron-free ammonia solution:* Contains approximately 10% w/w of NH_3 (iron-free). Dilute 425 ml of strong ammonia solution to 1000 ml.
4. *Iron standard solution (20 ppm Fe):* Dilute 1 volume of a 0.1726% w/v solution of ferric ammonium sulphate in 0.05 M sulphuric acid to 10 volumes with water. Contains iron in ferric state.

Experiment 04

Limit Test for Heavy Metals

Aim: To perform limit test for heavy metals for the given sample.

Requirements: Nessler cylinders, 1ml bulb pipette, dilute acetic acid, dilute ammonia solution, hydrogen sulphide solution, pH paper: 3-4 range, lead standard solution (20 ppm Pb), glass rods.

Principle: In this experiment, the test colour obtained by the reaction of heavy metal impurities with saturated solution of hydrogen sulphide is compared with standard colour obtained by the reaction of known quantity of lead with saturated solution of hydrogen sulphide.

$$Pb + H_2S \longrightarrow \underset{\text{colour (brown)}}{PbS\downarrow} + 2H^+$$

The precipitate of heavy metal sulphide formed gives colour. Dilute acetic acid and ammonia are used to maintain pH between 3.0 and 4.0 so that the precipitate formed is colloidal and uniform. The saturated solution of hydrogen sulphide has to be prepared freshly for the experiment. 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 I: It is used for the substance which gives a clear, colorless solution under specified conditions.

Method II: It is used for the substance which does not give a clear, colorless solution.

Method III: It is used for the substance which gives a clear, colorless solution in sodium hydroxide medium.

Procedure:

Test colour: Dissolve the given sample in 25 ml of water and transfer into a Nessler cylinder. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix. Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water, allow to stand for 5 minutes and view downwards over a white surface.

Standard colour: Pipette 1.0 ml of lead standard solution (20 ppm Pb) into a Nessler cylinder and dilute with water to 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix. Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water, allow to stand for 5 minutes and view downwards over a white surface.

Method I: It is used for the substance which gives a clear, colorless solution under specified conditions.

Test solution	Standard solution
The sample solution is prepared as per the monograph and 25 ml of solution is transferred into a Nessler's cylinder.	Transfer 1.0 ml of standard lead solution and dilute to 25 ml with water.
Add dilute ammonia or acetic acid solution to adjust the pH between 3 to 4	Add dilute ammonia or acetic acid solution to adjust the pH between 3 to 4
Dilute to 35 ml with distilled water and mix well.	Dilute to 35 ml with distilled water and mix well.
Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water.	Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water.
Allow to stand for 5 min and view downwards over a white background.	Allow to stand for 5 min and view downwards over a white background.

Method II: It is used for the substance which does not give a clear, colorless solution.

Test solution	Standard solution
Transfer the prescribed quantity of sample into a crucible.	Transfer 1.0 ml of standard lead solution and dilute to 25 ml with water.
Moisten the sample with sulphuric acid and ignite on a low flame until complete charring of the sample.	Add dilute ammonia or acetic acid solution to adjust the pH between 3 to 4

Table ***Contd…***

Test solution	Standard solution
Add 2 to 3 drops of nitric acid and heated to 500 ^{o}C and cool it.	Dilute to 35 ml with distilled water and mix well.
Then add 4 ml of dilute hydrochloric acid and digest for 2 min and evaporate to dryness. Then add 10 ml of dilute hydrochloric acid and digest the residue for two min.	Add 10ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water.
Neutralize with dilute ammonia solution and just acidified with acetic acid.	Allow to stand for 5 min and view downwards over a white background.
Adjust the pH 3 to 4, filter if necessary. Dilute to 35 ml with water.	
Add 10 ml of freshly prepared hydrogen sulphide solution. Dilute to 50 ml with water.	
Allow to stand for 5 min and view downwards over a white background.	

Method III: It is used for the substance which gives a clear, colorless solution in sodium hydroxide medium.

Test solution	Standard solution
The required quantity of sample is dissolved in 20 ml of water; add 5 ml of sodium hydroxide solution or the sample solution is prepared as per monograph.	The standard solution is prepared by using 1.0 ml of standard lead solution; add 5 ml of sodium hydroxide solution.
Make up to 50 ml with water.	Make up to 50 ml with water.
Add 5 drops of sodium sulphide solution mix well and kept aside for 5 min.	Add 5 drops of sodium sulphide solution mix well and kept aside for 5 min.
View downwards over a white background.	View downwards over a white background.

Observation:

If the color produced in the test solution is not more intense than that of standard solution, the sample complies with the standards of I.P or vice versa.

Test colour is not more intense than standard colour.

or

Test colour is more intense than standard colour.

Report/Result:

The given sample passes limit test for heavy metals.

or

The given sample fails limit test for heavy metals.

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.

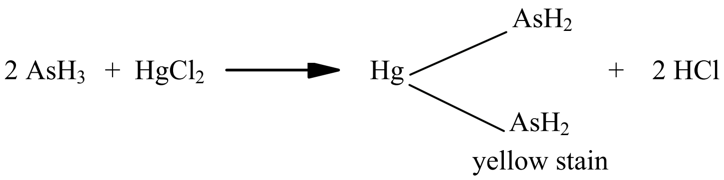
Experiment 05

Limit Test for Arsenic

Aim: To perform limit test for arsenic for the given sample.

Requirements: Arsenic apparatus, lead acetate cotton, mercuric chloride paper, 1 M potassium iodide solution, zinc dust (As T), water bath, arsenic standard solution (10 ppm As), 1 ml bulb pipette, 5 ml pipette, stannated hydrochloric acid.

Principle: In this experiment, the test stain obtained by the reaction of arsenic impurities in the form of arsine gas with mercuric chloride (paper) is compared with standard stain obtained by the reaction of known quantity of arsenic (in the form of arsine gas) with mercuric chloride.



A specially designed apparatus is used for the limit test. In addition to the above product $AsH(HgBr)_2$, $As(HgBr)$ and As_2Hg_3 are formed which also form yellow or brown stain on the mercuric chloride paper.

Here, Arsenic present is converted to arsenic acid in acid. The arsenic acid is then reduced to arsenous acid.

$$\underset{\text{Arsenic acid}}{H_3AsO_4} \longrightarrow \underset{\text{Arsenous acid}}{H_3AsO_3}$$

The nacent hydrogen produced by the reaction of zinc and hydrochloric acid reduces arsenous acid to arsine gas.

$$H_3AsO_3 + 3\,H_2 \longrightarrow \underset{\text{Arsine gas}}{AsH_3\uparrow} + 3\,H_2O$$

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.

$$Pb(CH_3COO)_2 + H_2S \longrightarrow PbS\downarrow + 2\,CH_3COOH$$

The method is called the Gutzeit method (modified)

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.

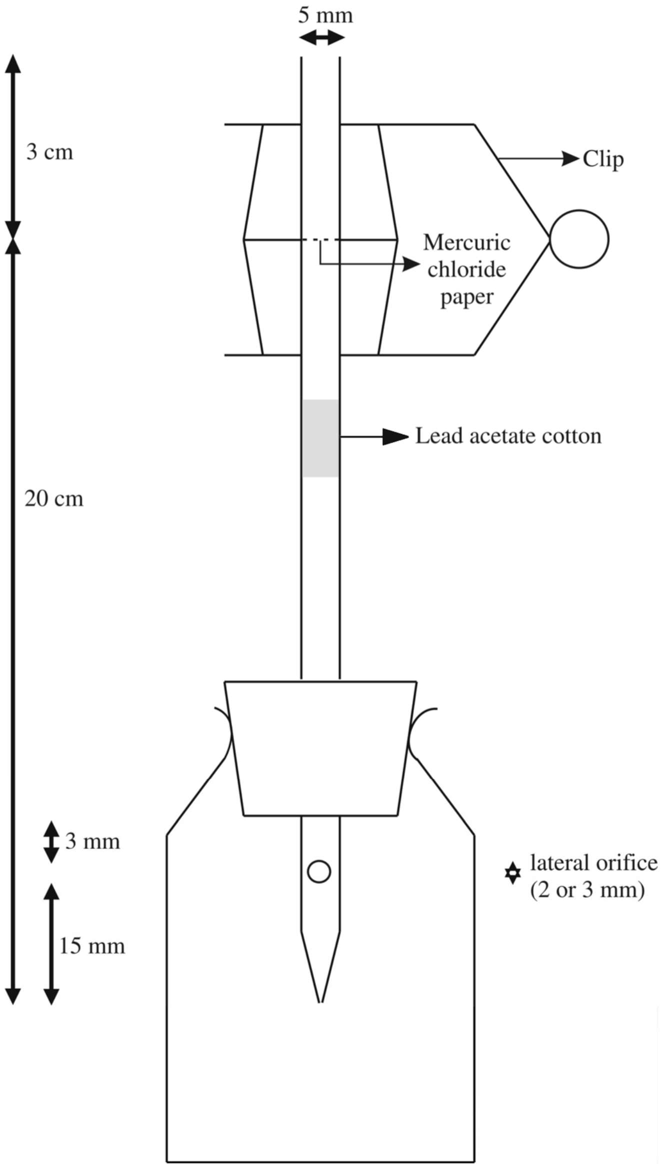
Stannous chloride present in stannated hydrochloric acid reduces arsenic (As^{5+}) to arsenous (As^{3+}). Hydrogen gas liberated also act as a carrier gas for arsine.

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.

Description of Apparatus:

The apparatus (see figure) 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 X 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 X 15 mm).

Diagram of Arsenic Apparatus:



Procedure:

Test stain: Dissolve the given sample in 50 ml water and add 10 ml of stannated hydrochloric acid and transfer into the arsenic apparatus bottle. Add 5 ml of 1 M potassium iodide and 10 g of zinc AsT. Immediately assemble the apparatus and immerse the bottle in a water bath at a temperature such that a uniform evolution of gas is maintained. After 40 minutes observe the stain produced on the mercuric chloride paper.

Standard stain: Transfer 1.0 ml of arsenic standard solution into an arsenic apparatus bottle and dilute to 50 ml with water. Add 10 ml of stannated hydrochloric acid. Add 5 ml of 1 M potassium iodide and 10 g of zinc AsT. Immediately assemble the apparatus and immerse the bottle in a water bath at a temperature such that a uniform evolution of gas is maintained. After 40 minutes observe the stain produced on the mercuric chloride paper.

Observation:

Test stain is not more intense than standard stain.

or

Test stain is more intense than standard stain.

Report/Result:

The given sample passes limit test for arsenic.

or

The given sample fails limit test for arsenic.

Preparation of Reagents:

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 *x*M may be prepared by dissolving 40*x* 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.
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°, 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.

Experiment 06

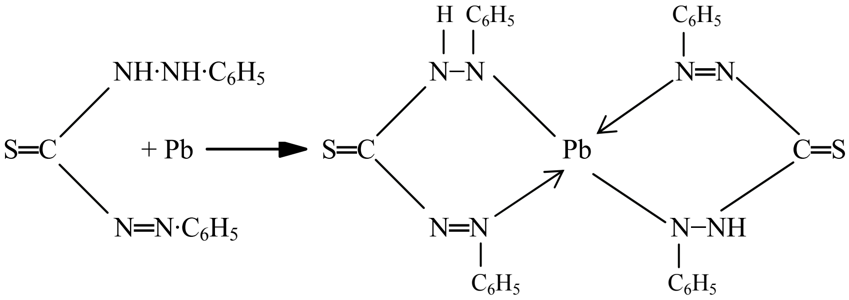
Limit Test for Lead

Aim: To perform limit test for lead for the given sample.

Requirements: Ammonium citrate solution Sp, hydroxyl amine hydrochloride solution Sp, phenol-red solution, strong ammonia solution, potassium cyanide solution Sp, dithizone extraction solution, 1% v/v solution of nitric acid, chloroform, dithizone standard solution, lead standard solution (1 ppm Pb), separating flasks, beakers, separating flask ring, test tubes.

Principle: In this experiment, the test colour in chloroform layer obtained by the reaction of lead impurities with diphenyl thiocarbazone (dithizone) is compared with standard colour in chloroform layer obtained by the reaction of known quantity of lead with diphenyl thiocarbazone (dithizone).

Dithizone in chloroform, extracts lead from alkaline aqueous solution as lead dithizone complex (violet in colour).



Lead dithionate complex

In this experiment, ammonium citrate, potassium cyanide, hydroxylamine hydrochloride are used to extract and discard any interfering metal ions (other than lead) at optimum pH in the form of complex.

The original dithizone has green colour in chloroform, thus lead-dithizone shows violet colour. Intensity of the colour depends upon the amount of lead in the solution. Here basically, the sample dissolved in water after adding the reagents as per the procedure are made to extract with dithizone extract solution. The dithizone extract solution is separated (in chloroform) from water layer and a fresh dithizone extract solution is added to the previously extracted sample solution and then re-extracted and then organic layer separated from aqueous layer. The organic layer i.e., di-thizone lead complex layer is combined with the first. The procedure of extraction is continued until freshly taken di-thizone extract solution on extraction with sample solution does not give any violet colour indicating that all the lead has been extracted from the sample. Now all the combined chloroform layers containing lead-thizone complex (violet) is extracted with an aqueous solution of nitric acid so that the lead is now extracted from chloroform layer into aqueous layer by forming lead nitrate (soluble in water). After extracting with nitric acid, the combined di-thizone extraction solutions can be discarded since the lead is no more in the organic layer. Now the aqueous solution is extracted with exactly 5 ml of dithizone standard solution, separated and compared with standard solution. One has to keep in mind that the volume of di-thizone extract solution for extraction need not be accurate but has to take exactly 5 ml of di-thizone standard solution for colour comparison.

Procedure:

Test colour: Dissolve the given sample (as specified in the monograph) in water and transfer into a separator, add 6 ml of ammonium citrate solution Sp. and 2 ml of hydroxylamine hydrochloride solution Sp. Add two drops of phenol red solution and make the solution just alkaline (red in colour) by the addition of strong ammonia solution. Cool the solution if necessary and add 2 ml of potassium cyanide solution Sp. Immediately extract the solution with several quantities, each of 5 ml of dithizone extraction solution, draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combined dithizone solutions for 30 seconds with 30 ml of a 1% v/v solution of nitric acid and discard the chloroform layer. Add to the acid solution exactly 5 ml of dithizone standard solution and shake for 30 seconds. Observe the colour of chloroform layer.

Standard colour: Transfer a volume of lead standard solution (1ppm Pb) equivalent to the amount of lead permitted in the substance being examined into a separator, add 6 ml of ammonium citrate solution Sp. and 2 ml of hydroxylamine hydrochloride solution Sp. Add two drops of phenol red solution and make the solution just alkaline (red in colour) by the addition of strong ammonia solution. Cool the solution if necessary and add 2 ml of potassium cyanide solution Sp. Immediately extract the solution with several quantities, each of 5 ml of dithizone extraction solution, draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combined dithizone solutions for 30 seconds with 30 ml of a 1% v/v solution of nitric acid and discard the chloroform layer. Add to the acid solution exactly 5 ml of dithizone standard solution and shake for 30 seconds. Observe the colour of chloroform layer.

Test solution	Standard solution
The required quantity of sample is dissolved in water and transferred into a separating funnel.	Transfer required amount of lead standard solution (1 ppm lead) equivalent to the amount of lead permitted in the substance being examined into a separating funnel
Add 6 ml of ammonium citrate solution (Sp).	Add 6 ml of ammonium citrate solution (Sp).
Add 2 ml of hydroxylamine hydrochloride solution (Sp), and add two drops of phenol red solution.	Add 2 ml of hydroxylamine hydrochloride solution (Sp), and add two drops of phenol red solution.
Make the solution just alkaline by the addition of strong ammonia solutions cool it if necessary.	Make the solution just alkaline by the addition of strong ammonia solution cool it if necessary.
Add 2 ml of potassium cyanide solution (Sp).	Add 2 ml of potassium cyanide solution (Sp).
Extract immediately with several quantities, each of 5 ml of dithizone extraction solution until it becomes green.	Extract immediately with several quantities, each of 5 ml of dithizone extraction solution until it becomes green.
Combine the dithizone extracts and shaken for 30 seconds with 30 ml of 1 % v/v solution of nitric acid and discard the chloroform layer. (Dithizone remains in chloroform layer, lead nitrate in aqueous layer).	Combine the dithizone extracts and shaken for 30 seconds with 30 ml of 1 % v/v solution of nitric acid and discard the chloroform layer (dithizone remains in chloroform layer, lead nitrate in aqueous layer).
To this acid solution add 5 ml of standard dithizone solution.	To this acid solution add 5 ml of standard dithizone solution.
Shake well for 30 min and observe the color of chloroform layer after separation.	Shake well for 30 min and observe the color of chloroform layer after separation.

Observation:

Test colour of the chloroform layer is not more intense than standard colour of the chloroform layer.

or

Test colour of the chloroform layer is more intense than standard colour of the chloroform layer.

Report/Result:

The given sample passes limit test for lead.

or

The given sample fails limit test for lead.

Preparation of Reagents:

1. *1% v/v nitric acid:* Dilute 1 vol of nitric acid to 100 volumes with water.
2. *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.
3. *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.

9. *Lead standard solution (100 ppm Pb):* Dilute 1 volume of lead standard solution (0.1% Pb) to 10 volumes with water.
10. *Potassium cyanide solution Sp:* Dissolve 50 g of potassium cyanide in sufficient water to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml of dithizone extraction solution until the dithizone solution retains its orange green colour. Extract any dithizone remaining in the cyanide solution by shaking with chloroform. Dilute this cyanide solution with sufficient water to produce a solution containing 10 g of potassium cyanide in each 100 ml.
11. *Strong ammonia solution:* (ammonia solution, strong) Contains 25.0% w/w of NH_3 (limits, 24.5 to 25.5); wt. per ml about 0.91 g; strength about 13.5 M. Store in well-closed containers in a cool place.

    Note: Use directly the sample in case of liquids.

Experiment 07

Limit Test for Chlorides for Water Insoluble Substances

Aim: To perform limit test for chlorides for water insoluble activated charcoal.

Requirements: Nessler cylinders, glass rod, measuring cylinders, 1ml bulb pipette, 10 ml bulb pipette, dilute nitric acid, 0.1 M silver nitrate, chloride standard solution (25 ppm Cl), test sample (activated charcoal).

Principle: In this experiment, the test opalescence obtained by the reaction of chloride impurities with silver nitrate is compared with standard opalescence obtained by the reaction of known quantity of chloride with silver nitrate. Dilute nitric acid is used to dissolve other impurities if present.

$$Cl^- + AgNO_3 \xrightarrow{\text{Dil . }HNO_3} \underset{\text{opalescence}}{AgCl\downarrow} + NO_3^-$$

The precipitate silver chloride formed is insoluble in dilute nitric acid and gives opalescence.

Here, the main objective is to check for chloride impurity in charcoal and not as such charcoal. Usually chloride salts are soluble in water. When charcoal is treated with water, chloride salt gets dissolved, where as charcoal does not. Hence, the filterate of the suspension is used for limit test for chloride.

Procedure:

Test opalescence: Boil 3.0 g of the activated charcoal with 75 ml water for 5 minutes, cool. Dilute to 100.0 ml with water and filter. Transfer 6.0 ml of the filterate to a Nessler cylinder. Add 10 ml of dilute nitric acid, dilute to 50 ml with water. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light. View transversely against a black background.

Standard opalescence: Transfer 10.0 ml of chloride standard solution (25 ppm Cl) into a Nessler cylinder and add 5 ml of water. Add 10 ml of dilute nitric acid, dilute to 50 ml with water. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light. View transversely against a black background.

Observation:

Test opalescence is not more intense than standard opalescence.

or

Test opalescence is more intense than standard opalescence.

Report/Result:

The given sample passes limit test for chlorides.

or

The given sample fails limit test for chlorides.

Preparation of Reagents:

1. *0.1 M silver nitrate:* Dissolve 17.0 g of silver nitrate in sufficient water to 1000 ml. (Store in light-resistant containers)
2. *Chloride standard solution (25 ppm Cl):* Dilute 5 volumes of a 0.0824% w/v of sodium chloride to 100 volumes with water.
3. *Dilute nitric acid:* Contains approximately 10% w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Experiment 08

Limit Test for Chlorides for Coloured Substances

Aim: To perform limit test for chlorides for the given sample (potassium permanganate).

Requirements: Nessler cylinders, glass rod, measuring cylinders, 1ml bulb pipette, 10 ml bulb pipette, dilute nitric acid, 0.1 M silver nitrate, chloride standard solution (25 ppm Cl), test sample, ethanol (95%).

Principle: In this experiment, the test opalescence obtained by the reaction of chloride impurities with silver nitrate is compared with standard opalescence obtained by the reaction of known quantity of chloride with silver nitrate. Dilute nitric acid is used to dissolve other impurities if present.

$$Cl^{-} + AgNO_3 \xrightarrow{\text{Dil . HNO}_3} \underset{\text{opalescence}}{AgCl\downarrow} + NO_3^{-}$$

The precipitate silver chloride formed is insoluble in dilute nitric acid and gives opalescence.

Potassium permanganate in water possess purple colour. If limit test for chlorides is performed directly with potassium permanganate solution, the opalescence is not clearly observed due to colour of solution. Hence, potassium permanganate having chloride impurities is made to react with ethanol during which acetic acid (oxidation of ethanol by potassium permanganate) and manganese dioxide (reduction of potassium permanganate) are produced. The resultant manganese dioxide suspension which on filteration, the filterate is colour less having the chloride impurity in it. This colour less filterate is used for the limit test for chloride.

$$KMnO_4 + CH_3CH_2OH \longrightarrow MnO_2\downarrow + CH_3COOH + K^{+}$$

Procedure:

Test opalescence: Dissolve 1.5 g of the given sample in 50 ml of water, heat on a water-bath and add gradually 6 ml of ethanol (95%). Cool. Dilute to 60 ml with water and filter. Transfer 40 ml of the filterate to a Nessler cylinder. Add 10 ml of dilute nitric acid. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light. View transversely against a black background.

Standard opalescence: Transfer 10.0 ml of chloride standard solution (25 ppm Cl) into a Nessler cylinder and add 5 ml of water. Add 10 ml of dilute nitric acid, dilute to 50 ml with water. Add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light. View transversely against a black background.

Test solution	Standard solution
Transfer 1.5gm of the sample into a 100ml beaker and add 50ml of distilled water. Heat it on a water bath.	Transfer 10.0 ml of standard chloride solution (25 ppm) in to a Nessler's cylinder and add 5 ml water.
To this, slowly add 6 ml of ethyl alcohol. Dilute to 60 ml with water. Cool the solution, filter and then collect the filtrate.	Add 10 ml of dilute nitric acid, dilute to 50 ml with water.
Transfer 40ml of filtrate into a Nessler's cylinder and add 10ml of dilute nitric acid. Add 1ml of 0.1M silver nitrate solution.	Add 1 ml of 0.1 M silver nitrate solution.
Stir immediately with a glass rod and allowed to stand for 5 min, protected from light and viewed transversely against a black background.	Stir immediately with a glass rod and allowed to stand for 5 min, protected from light and viewed transversely against a black background.

Observation:

Test opalescence is not more intense than standard opalescence.

or

Test opalescence is more intense than standard opalescence.

Report/Result:

The given sample passes limit test for chlorides.

or

The given sample fails limit test for chlorides.

Preparation of Reagents:

1. *0.1 M silver nitrate*: Dissolve 17.0 g of silver nitrate in sufficient water to 1000 ml. (Store in light-resistant containers)
2. *Chloride standard solution (25 ppm Cl):* Dilute 5 volumes of a 0.0824% w/v of sodium chloride to 100 volumes with water.
3. *Dilute nitric acid:* Contains approximately 10% w/w of HNO_3.

   Dilute 106 ml of nitric acid to 1000 ml with water.

Experiment 09

Limit Test for Chlorides in Sodium Benzoate

Aim: To perform the limit test for chlorides for the given sample of sodium benzoate and report its compliance /non compliance with standards of I.P.

Requirements:

The given sample, 0.1M silver nitrate solution, dilute nitric acid, distilled water, standard chloride solution (25 ppm) and sodium carbonate solution (0.5 M), Nessler's cylinder, glass rod, bulb pipette (1ml and 10 ml), standard flask and measuring cylinders.

Principle: Sodium benzoate is insoluble in water. If any chloride impurities are present in the sample, they are soluble in water and gives the opalescence when treated with silver nitrate. This test is performed by heating the sample with sodium carbonate solution until charring occurs and dissolve the residue in dilute nitric acid and water. The resulting mixture is filtered and the filtrate obtained is used for performing the limit test.

Limit test for chloride is based upon the reaction of chloride impurities with silver nitrate in the presence of dilute nitric acid. The turbidity/opalescence produced in the test solution is compared with the standard turbidity/opalescence produced or obtained by the reaction of known quantity of chloride with silver nitrate.

$$Cl^- + AgNO_3 \xrightarrow{\text{Dilute } HNO_3} \underset{\text{opalescence/Turbidity}}{AgCl\downarrow} + NO_3^-$$

$$Na_2CO_3 + 2Cl^- + 2H^+ \longrightarrow 2NaCl + H_2O + CO_2$$

The silver chloride precipitate formed is insoluble in dilute nitric acid which gives turbidity/opalescence.

Reasons: 1.Dilute nitric acid is used to dissolve other impurities if present.

Procedure:

Test solution	Standard solution
Transfer 0.33 gm of sample in to a beaker. To this add 5 ml of 0.5 M sodium carbonate solution.	Transfer 10 ml of standard chloride solution (25 ppm) in to a Nessler's cylinder and add 5 ml water.

Table ***Contd…***

Test solution	Standard solution
Evaporate to dry the mixture, heat the residue at below 400 ^{o}C until complete charring occurs. Extract the residue with 10 ml of water and 12 ml of dilute nitric acid. Filter the extract, collect the filtrate. Transfer the filtrate in to a Nessler's cylinder. Add 10 ml of dilute nitric acid, dilute to 50 ml with water.	Add 10 ml of dilute nitric acid, dilute to 50 ml with water.
Add 1 ml of 0.1 M silver nitrate solution.	Add 1 ml of 0.1 M silver nitrate solution.
Stir immediately with a glass rod and allowed to stand for 5 mins, protected from light and viewed transversely against a black background.	Stir immediately with a glass rod and allowed to stand for 5 mins, protected from light and viewed transversely against a black background.

Observation and Inference:

If the opalescence /turbidity produced in the test solution is less than that of the standard solution, the given sample complies with the standards of I.P. or vice versa.

Report: The given sample complies / not complies with the standards of I.P.

Preparation of Reagents:

1. **0.1 M $AgNO_3$ solution**

   Dissolve 17 gm of silver nitrate in sufficient amount of distilled water to produce 1000 ml and stored in light resistant container.

2. **Dilute Nitric acid (10% w/w of HNO_3)**

   Dissolve 106 ml of nitric acid in distilled water to produce 1000ml.

3. **Chloride standard solution (25 ppm chloride)**

   Dissolve 0.0824 gm of sodium chloride in 100ml of distilled water (stock solution). Dilute 3ml of the above stock solution to 100ml with distilled water.

4. **Preparation of 0.5 M Sodium carbonate solution**

   Dissolve 143.75 gm of sodium carbonate in 1000 ml of water.

Experiment 10

Limit Test for Chlorides in Sodium Bicarbonate

Aim: To perform the limit test for chlorides for the given sample of sodium bicarbonate and report its compliance /non compliance with standards of I.P.

Requirements:

Test sample, 0.1M silver nitrate solution, dilute nitric acid, nitric acid (conc.) distilled water and standard chloride solution (25 ppm), Nessler's cylinder, glass rod, bulb pipette (1ml and 10 ml), standard flask and measuring cylinders.

Principle:

Limit test for chlorides is based upon the reaction of chloride impurities with silver nitrate in the presence of dilute nitric acid. The turbidity/opalescence produced in the test solution is compared with the standard turbidity/ opalescence produced or obtained by the reaction of known quantity of chloride with silver nitrate.

$$Cl^{-} \quad + \quad AgNO_3 \xrightarrow{\text{Dilute } HNO_3} \underset{\text{opalescence/Turbidity}}{AgCl\downarrow} \quad + \quad NO_3^{-}$$

The silver chloride precipitate formed is insoluble in dilute nitric acid which gives turbidity/opalescence.

Reasons: 1.Dilute nitric acid is used to dissolve other impurities if present.

Procedure:

Test solution	Standard solution
Dissolve 1.25g of sample in 15 ml water and transfer in to Nessler's cylinder.	Transfer 10 ml of standard chloride solution (25 ppm) in to a Nessler's cylinder and add 5 ml water.
Then add 2 ml of nitric acid, dilute to 50 ml with water.	Add 10 ml of dilute nitric acid, dilute to 50 ml with water.
Add 1 ml of 0.1 M silver nitrate solution.	Add 1 ml of 0.1 M silver nitrate solution.
Stir immediately with a glass rod and allowed to stand for 5 mins, protected from light and viewed transversely against a black background.	Stir immediately with a glass rod and allowed to stand for 5 mins, protected from light and viewed transversely against a black background.

Observation and Inference:

If the opalescence /turbidity produced in the test solution is less than that of the standard solution, the given sample complies with the standards of I.P. or vice versa.

Report: The given sample complies / not complies with the standards of I.P.

Preparation of Reagents:

1. **0.1 M $AgNO_3$ solution**

   Dissolve 17gm of silver nitrate in sufficient amount of distilled water to produce 1000 ml and stored in light resistant container.

2. **Dilute Nitric acid (10% w/w of HNO_3)**

   Dissolve 106 ml of nitric acid in distilled water to produce 1000ml.

3. **Chloride standard solution (25 ppm chloride)**

   Dissolve 0.0824 gm of sodium chloride in 100ml of distilled water (stock solution). Dilute 5 ml of the above stock solution to 100 ml with distilled water.