UNIT – I IMPURITIES IN PHARMACEUTICAL SUBSTANCES

POINTS TO BE COVERED IN THIS TOPIC

- HISTORY OF PHARMACOPOEIA
- SOURCES AND TYPES OF IMPURITIES
- CHLORIDE
- SULPHATE
- IRON
- ARSENIC
- LEAD
- HEAVY METALS
- MODIFIED LIMIT TEST FOR CHLORIDE AND SULPHATE

HISTORY OF PHARMACOPOEIA

➢ PHARMACOPOEIA

- The word derives from the ancient **Greek** word **pharmacon** means drug and **poeia** to make.
- It is a legally **binding collection** , prepared by a **national or regional authority** and contains list of **medicinal substances** , **crude drug** and formulas for making **preparation** from them.

❖ THE PHARMACOPEIA CONTAIN

- **List of drug** and other related substances
- **Sources**
- **Description**
- **Tests**
- **Formulas** for preparation actions
- **Uses**
- **Doses**
- **Storage condition**

❖ DIFFERENT TYPES OF PHARMACOPOEIA

- **United states** pharmacopeia
- **Indian** pharmacopeia
- **British** pharmacopeia
- **German** pharmacopeia
- **Mexican** pharmacopeia
- **French** pharmacopeia
- **Japanese** pharmacopeia

❖ INDIAN PHARMACOPEIA

- **Indian pharmacopeia** commission is an **autonomous institution** of the ministry of **health and family welfare** which sets standards for all drugs that are **manufactured sold** and consumed in India.

EDITION	YEAR	SUPPLEMENT / ADDENDUM
1st edition	1955	Supplement 1960
2nd edition	1966	Supplement 1975 Addendum 1989
3rd edition	1985	Addendum 1989 Addendum 1991
4th edition	1996	Addendum 2000 Vet supplement 2000 Addendum 2002 Addendum 2005
5th edition	2007	Addendum 2008
6th edition	2010	Addendum 2012
7th edition	2014	Addendum 2015 Addendum 2016
8th edition	2018	Addendum 2019

✓ Salient features of first edition of Indian Pharmacopoeia (1955)

- Covers **986 monographs.**
- **Titles of monograph** in Latin language.
- **Weight and measures** in metric system.
- **Doses expressed** in both **metric and English system.**
- **List of preparations** given at the end of some of the monographs.
- **Abbreviated titles used.**
- Descriptive terms used for **solubility instead** of **exact solubility.**

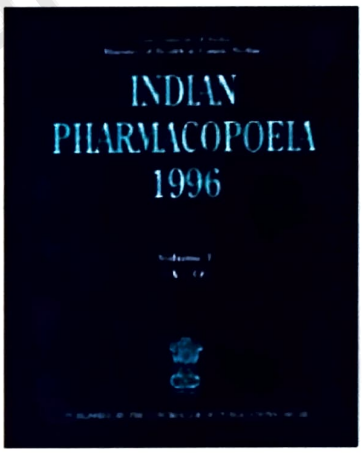
✓ Salient features of second edition of Indian Pharmacopoeia (1966):

- Titles of monographs changed from **Latin to English.**
- **Solubility expressed** in parts of **solvent per unit part of solute.**
- **93 new monographs** were added.
- New **analytical techniques** had been included.
- Test for **sterility** had been modified to **detect fungi.**

✓ Salient features of third edition of Indian Pharmacopoeia (1985):

- **260 new monographs** were added and **450 were amended.**
- New analytical techniques (**Flame photometry, Electrophoresis, Fluorometry etc.**) had been introduced.
- **Dissolution** had been introduced.
- **Microbial limit tests** being prescribed for liquid preparation.
- **Gas liquid chromatography** had been recognized.
- Determination of **viscosity** been modified involving use of **Ostwald viscometer.**

✓ Salient features of fourth edition of Indian Pharmacopoeia (1996)

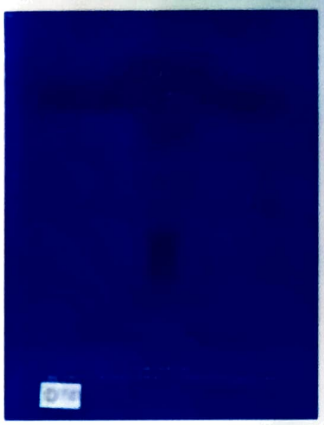


- Contains **1149 monographs** and **123 appendices** in two volumes.
- **Computer generated** structural formulae used.
- **Infrared** and **Ultra Violet** absorption **spectrophotometric tests** for identification of drug were added.
- **Included 294** new monographs and **110 monographs** were deleted.
- **High Pressure Liquid Chromatography (HPLC)** had been used as analytical method.
- **Bacterial endotoxin** test were introduced.
- The **veterinary supplement** of I.P. 1996 contains **208 monographs** and four appendices.

✓ Salient features of fifth edition of Indian Pharmacopoeia (2007):

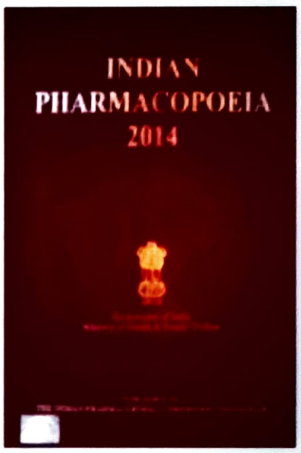
- Presented in **three volumes.**
- Volume **one contains general notices, structure of IPC,** acknowledgements.
- Volume **two and three** contains **general monographs** on dosage forms, **drug substances and pharmaceutical aid**

✓ Salient features of sixth edition of Indian Pharmacopoeia (2010):



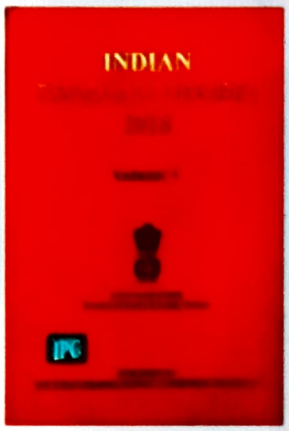
- Consists of **three volumes.**
- Volume one contains **Notices, preface, structure of IPC, general chapters**
- **Volume two** contains general monographs **on dosage form, drug and pharmaceutical aid** (A to M).
- **Volume three** contains **general monographs** on **dosage form,** drug and **pharmaceutical aid** (N to Z)
- **Microbial contamination** chapter updated
- New chapter on **Liposomal products** is also added.

✓ Salient features of seventh edition of Indian Pharmacopoeia (2014):



- **Published in 2014** and presented in **four volumes**
- **Contain 2567 monographs** of drugs out of which **577** are new monographs.
- Introduced **19 radiopharmaceutical monographs** for the first time
- **10 antibiotic monographs, 31 herbal** monographs, 5 vaccine and **immunosera** for human use, **6 insulin products** and **7 biotechnological** products with 19 new general chapters were included.

✓ Salient features of eight edition of Indian Pharmacopoeia (2014)



- The use of **chromatographic methods** has been greatly extended to cope with the need for **more specificity** in assays and in **particular,** in **assessing the nature** and extent of impurities in ingredients and products.

- **Pyrogen tests** have been replaced by **Bacterial Endotoxin** tests (BET) in **parenteral preparations** and other monographs
- 53 New **Fixed-Dose Combination (FDC)** combination monographs have been included, out of which **25 FDC monographs** are not available in any **Pharmacopoeia.**
- General Chapters on **Volumetric Glassware, Conductivity, Dissolution test, Disintegration test, Dimensions of Hard Gelatin Capsule Shells** etc. have been revised.
- **Maintenance, Identification, Preservation and Disposal** of Microorganism have been revised.

SOURCES AND TYPES OF IMPURITIES

➢ IMPURITY

- Means presence of **unwanted foreign particle** other than active drugs which may be or may **not be toxic** and is found in **pharmaceutical** substances.

❖ SOURCES OF IMPURITY

- The various **sources of impurities** in **pharmaceutical substances** are as follows

✓ Raw material employed in manufacture

- The **raw materials** , from which these are prepared , often contain **impurities.**
- It is therefore necessary to **employ pure chemicals** and substances as **raw materials**
- Example :-
- Presence of **tin , lead , silver copper , cobalt** and **gold in bismuth salts.**
- **Rock salt** contains small amounts of **calcium sulfate** and **magnesium chloride.** So **sodium chloride** prepared from **rock salt** will almost contains trace of **calcium and magnesium** compounds as impurity.

✓ **Reagent employed in the manufacturing process**

- If **reagents** are employed in the **manufacturing process** are not **completely removed** by washing , these reagents may be present in final products.
- Example :- **magnesium impurities** are found in **calcium minerals, aluminum** ores are usually accompanied by **alkali and alkaline earth compounds.**

✓ **Solvents**

- Water is a **common solvent** in **large scale manufacturing** of pharmaceuticals .
- This can give rise to **trace impurities** such as **sodium , calcium magnesium , carbonate , chloride and sulfate ions.**
- This impurities can be avoided by **using purified water.**

✓ **Action of reagents on reaction vessels**

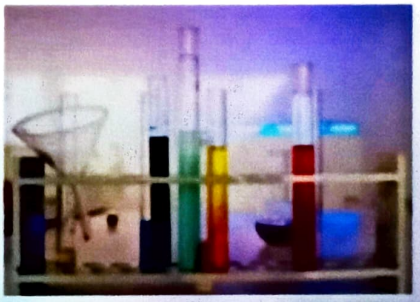
- Reaction vessels used in the **manufacturing process** may be **metallic such as iron , cast iron , galvanized iron , copper , silver aluminum , nickel , zinc and lead.**

✓ **Atmospheric contamination during manufacturing process**

- **Atmosphere** may contain dust (Sulphur , aluminum oxide , silica , soot etc.) and **some gases** like **carbon dioxide , sulphur dioxide, arsine and hydrogen sulphide.**

LIMIT TEST

- **Limit test** is defined as **quantitative or semi-quantitative test** designed to identify and **control small quantity** of impurity which is likely to be present in the substance



LIMIT TEST OF CHLORIDE

- 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.

$$NaCl + AgNO3 \xrightarrow{HNO_3} AgCl + NaNO_3$$

❖ 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 0.05845 % W/V solution** of sodium chloride in **Nessler cylinder.**
Add **1ml of nitric acid**	Add **1ml 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 solutior **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**

❖ REASONS

- Nitric acid is added in the limit test of chloride to make solution acidic and helps silver chloride precipitate to make solution turbid at the end of process.

LIMIT TEST OF SULPHATE

❖ PRINCIPLE

- Limit test of **sulphate** is based on the **reaction of soluble sulphate** with **barium chloride** in presence of **dilute hydrochloric acid** to form **barium sulphate**
- Which **appears as solid particles(turbidity)** in the solution
- The turbidity produced is **compared with the standard solution.**
- **Barium sulphate reagent** contains **barium chloride**, **sulphate free alcohol** and small amount of **potassium sulpahte.**
- **Alcohol prevents super saturation** and **more uniform turbidity** develops.

❖ CHEMICAL REACTION

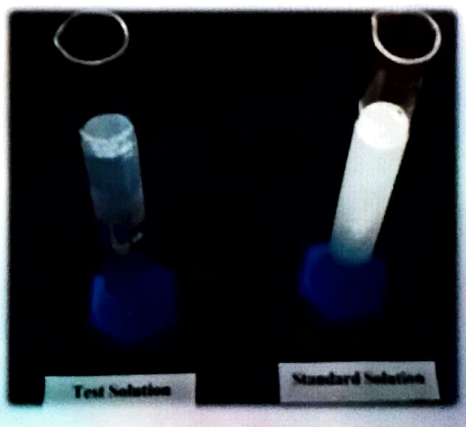
$$SO_4^{2-} + BaCl_2 \xrightarrow{HCl} BaSO_4 + KCl$$

❖ 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 **sulphate** and **vice-versa**

❖ REASONS

- **Hydrochloric acid** helps to **make solution acidic.**
- **Potassium sulphate** is used to **increase the sensitivity** of the test by giving ionic concentration in the reagent
- **Alcohol helps** to **prevent super saturation.**



LIMIT TEST OF IRON

❖ PRINCIPLE

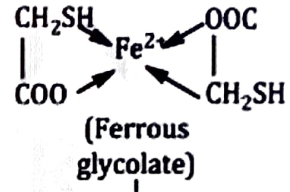
- Limit test of Iron is based on the reaction of **iron in Ammonical solution** with **thioglycollic acid** in **presence of citric acid** to form **iron thioglycolate** which is **pale pink to deep reddish purple in color**
- **Ferric iron** is reduced to **ferrous iron** by the **thioglycollic acid** and the compound produced is **ferrous thioglycollate**
- **Citric acid forms** a soluble complex **with iron** and **prevents its precipitation** by ammonia as ferrous hydroxide
- The **colour develops** only in the **presence of alkali.**
- The **colour is due to** the **formation co-ordination compound, ferrous thioglycollate** which is **stable in the absence of air** bud fades in air due to oxidation.
- Therefore, the **colour should be compared immediately** after the **time** allowed for full development of colour is over

❖ CHEMICAL REACTION

$2Fe^{3+}$ + $2\,SHCH_2COOH$ ⟶ $2Fe^{2+}$ + $2\,SHCH_2COOH$

Ferric ion — Thioglycollic acid — Ferrous ion

Citric acid, Ammonical alkaline Solution

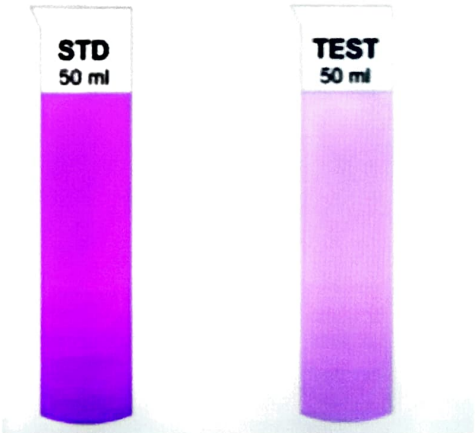


(Ferrous glycolate)

(Purple coloured Complex)

❖ OBSERVATION

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



❖ REASONS

1. **Citric acid helps precipitation** of **iron by ammonia** by forming a complex with it.
2. **Thioglycolic acid** helps **to oxidize iron (II) to iron (III).**
3. **Ammonia** to make **solution alkaline**

LIMIT TEST OF ARSENIC

❖ PRINCIPLE

- The principle is based on **Gutzeit Test** wherein, all **arsenic present** is duly converted into **arsine gas** (AsH_3) by subjecting it to reduction **with zinc and hydrochloric acid.**
- Limit test of Arsenic is based on the reaction of **arsenic gas** with **hydrogen ion** to form **yellow stain on mercuric chloride paper** in presence of **reducing agents** like **potassium iodide.**
- The **intensity of the stain** is proportional to the **amount of arsenic present**
- The **stain is compared** with that produced from a **known amount of arsenic**
- The **IP prescribes the limits** for the **presence of arsenic (NMT 2 ppm)** as an impurity in various pharmaceutical substances
- **Apparatus** used for arsenic limit test is called **Gutzeit apparatus.**

❖ CHEMICAL REACTION

$$Zn + 2HCl \longrightarrow ZnCl_2 + 2H^+$$

$$H_3AsO_4 + 2[H] \longrightarrow H_3AsO_3 + H_2O$$

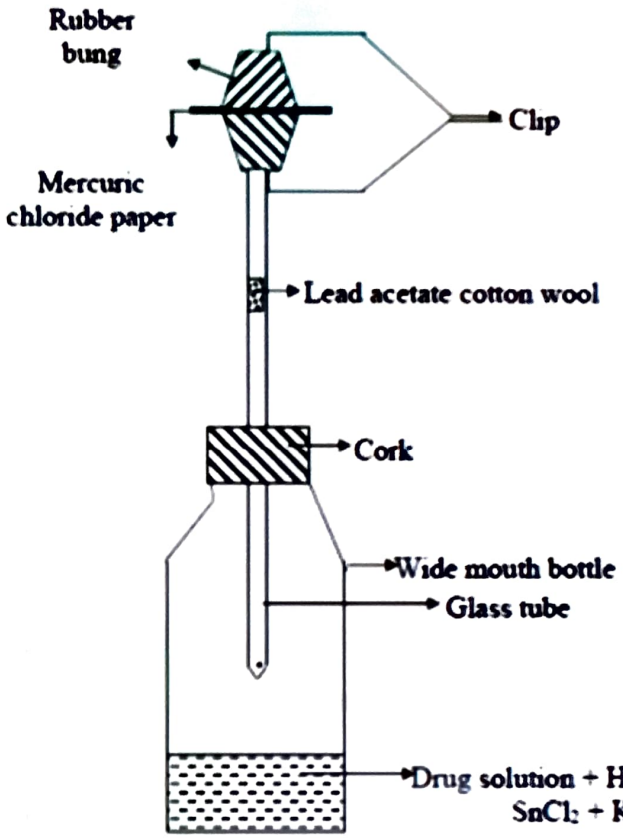
Arsenic acid → Arsenious acid

$$H_3AsO_3 + 3H_2 \longrightarrow AsH_3 + 3H_2O$$

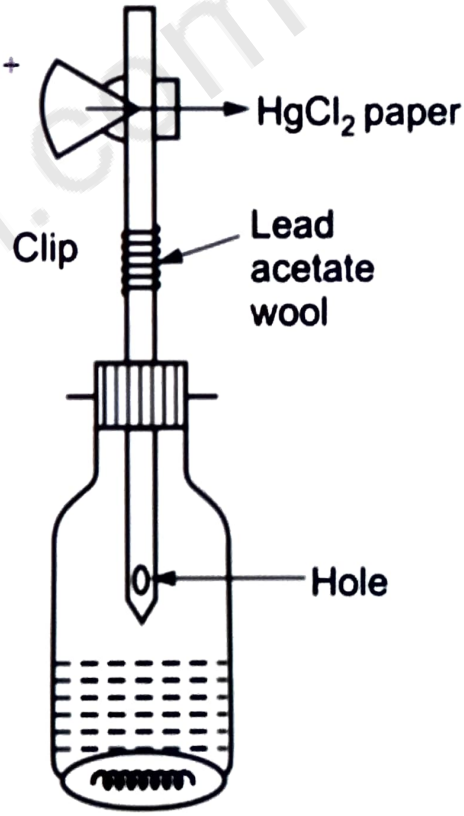
Arsenious acid → Arsine gas

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

Arsine gas + Mercuric chloride → Yellow stain



Gutzeit Test Appratus



❖ OBSERVATION

- If the **sample** show stain **lesser intensity** than that of the **standard stain** the **sample passed** the limit test for **arsenic as per IP**

❖ REASONS

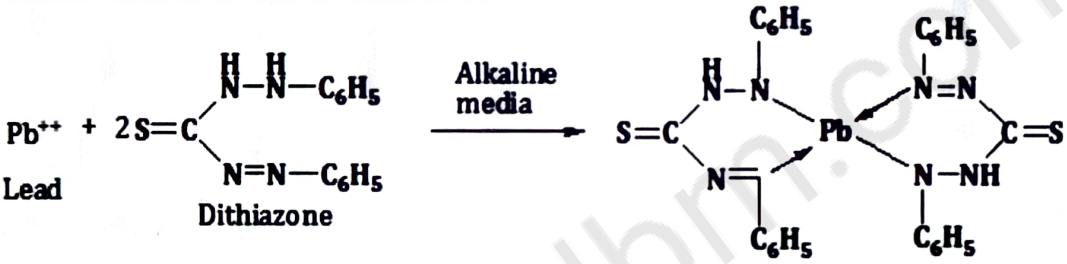
1. **Stannous chloride** is used for complete **evolution of arsine.**
2. **Zinc, potassium iodide** and **stannous chloride** is used as a **reducing agent.**
3. **Hydrochloric acid** is used to make the **solution acidic.**
4. **Lead acetate pledger** or papers are **used to trap any hydrogen sulphide** which may be evolved **along with arsine.**

LIMIT TEST OF LEAD

❖ PRINCIPLE

- Limit Test for Lead is based upon the chemical reaction between **lead** and **diphenyl thiocarbazone (dithizone)** in an **alkaline solution** to form **lead dithizone**, which is **red.**
- **Dithizone itself is green** in colour and the **lead dithizone** formed is **violet** in colour. Thus, the **net resultant colour** of the solution **becomes red.**
- To avoid **interference by other metals** and **make the pH optimum**, reagents like **ammonium citrate**, **KCN**, and **$NH_2OH.HCl$** is employed.

❖ CHEMICAL REACTION



lead-dithiazone complex

❖ OBSERVATION

- The **intensity of the color of the complex** depends on the **amount of lead** in the solution.
- The **color produces** in the **sample solution** should **not be greater** than the **standard solution.**

LIMIT TEST OF HEAVY METALS

❖ PRINCIPLE

- The limit test for **heavy metals** is **based on the reaction** of **metallic impurities** with **hydrogen sulfide** in **acidic medium**; the reaction **product will be the sulphides** of the respective metals
- In **acidic media**, it **produces reddish / black colour** with **Hydrogen sulphide** which is compared with **standard lead nitrate solution.**

- 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**
- Metals that **response to** this test are **lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper,** and **molybdenum**

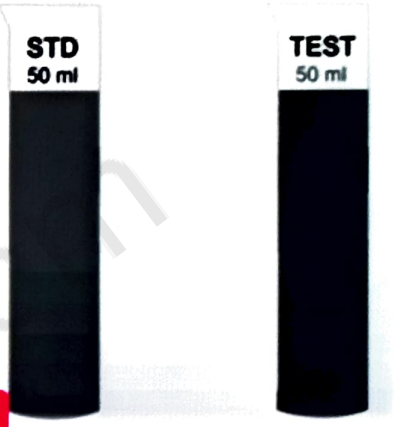
❖ CHEMICAL REACTION

Heavy Metal + H_2S ⟶ Sulphide of Heavy metal + $2H^+$

Brownish colour

❖ 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



MODIFIED LIMIT TEST FOR CHLORIDE

❖ PRINCIPLE :-

- In chloride limit tests , precipitation is used to **measure the concentration.**
- In the **presence of dilute nitric acid**, **chlorides precipitate** from **soluble chloride** when **silver nitrate reacts** with soluble chloride to produce **silver chloride**, the form of which appears as solid particles in the solution.
- Based on how much **chloride is present** in the test substance , the intensity of **turbidity is affected.**

❖ PROCEDURE

- The **limit test** for chloride has been modified within the context of the **preparation of standard solutions.**
- In the past , the **chlorine solution** was prepared through the **dissolution of sodium chloride** , but now it has been modified by substituting sodium chloride for **hydrochloric acid.**

$HCL + AgNO3 \rightarrow AgCl + HNO_3$

❖ CONCLUSION

- When the **opalescence produced** in the sample solution is lower than that produced in the **standard solution,** the sample will pass the limitation test for **chloride and vice versa.**

MODIFIED LIMIT TEST FOR SULFATE

❖ PRINCIPLE

- The **limit test** for **sulfates** uses the precipitation method as its basic principle.
- As a result of **reacting with barium chloride** in the presence of **hydrochloric acid,** only sulfate precipitates as other **acid radicals** do **not react with barium chloride** as **hydrochloride acid prevents** the reaction of different acid radicals with barium chloride.

$$SO_4(2-) + BaCl_2(HCl) \rightarrow BaSO_4 + 2Cl^-$$

❖ REAGENT PREPARATIONS

✓ **Barium sulfate reagent**

- Making a **0.05 M barium chloride solution** requires dissolving **12 grams of barium chloride** in 1000 ml of water.
- **Add 55 ml water , 20 ml alcohol,** and 5 ml 0.0181 % w/v potassium sulfate solution to 15 ml of the prepared solution. Makeup the **volume up to 100 ml.**

✓ **Standard potassium sulfate solution**

- The volume of **K_2SO_4 and** water was made up to **100 ml using 0.1089g of K_2SO_4** accurately weighed.

❖ TEST SOLUTION

✓ **Sodium chloride – 20 ml of water** should contain **2 grams of sodium chloride.**

✓ **Sodium bicarbonate –** in **small quantities** of water , **dissolve 2 grams** of **sodium bicarbonate.**

❖ PROCEDURE

- **Limit tests** for **sulfate** have undergone an **extensive modification.**
- By doing so, it **eliminated** the need for **barium sulfate reagents.**
- While **turbidity** is comparable through the use of **alcohol and barium chloride** the method still **uses alcohol.**