CHAPTER 1

Introduction to Pharmaceutical Chemistry

Introduction to Pharmaceutical Chemistry

Pharmaceutical chemistry is concerned with the drug design and synthesis of biologically active molecules/compound. The aim is to gain new chemical molecules that could enable the discovery of new pharmaceuticals or optimize already known drug structures, thereby to expand the portfolio of chemical drugs. It includes design and production of compounds that can be used for the prevention, treatment or cure of human and animal diseases. Medicinal chemistry includes the study of already existing drugs, of their biological properties and their structure-activity relationships. Pharmaceutical chemistry also includes other branches of study such as pharmacokinetics, pharmacodynamics, and drug metabolism.

Scope of Pharmaceutical Chemistry

Pharmaceutical Chemistry includes about the processes of drug development and distribution. The pharmaceutical industry is expanding day by day with the growth of medical fields worldwide. The pharmaceutical industry encompasses a wide and varied range of specialties, but one consistent element is the role of chemistry in each part of the pharmaceutical sciences. In the following areas, opportunities pertaining to pharmaceutical chemistry prevail:

(i) **Organic Chemistry:** Organic chemistry plays an important role in the pharmaceutical industry whereby knowledge of organic compounds is used to the discovery and development of new medicines.

(ii) **Computational Chemistry:** Computational chemistry is a specialty that contributes to the design of drugs as well as the drug discovery process by helping to design and study molecular structures and chemical compounds that are used as the foundation of new medicines.

(iii) **Analytical Chemistry:** Analytical chemistry includes pharmaceutical quality assurance and quality control ensuring the safety, stability, and efficacy of drugs and medicines. There is also a strong connection between analytic chemistry and high-performance liquid chromatography (HPLC), one of the most important analytical procedures in the drug development process. Their ability to perform complex analytical processes coupled with the skill set of managing hands-on testing.

(iv) **Miscellaneous:** The demand for drugs and medicines is increasing with advancements in discoveries, treatments and increasing illnesses. With the latest technologies and trends, the opportunities are also increasing with each passing year in the following areas related to pharmaceutical chemistry:

- Pharmaceutical Industry or factories
- Research Centers
- Laboratories for testing and analytical techniques
- Manufacturing industries
- Food industries
- Product marketing agencies
- Health Centers
- Clinics
- Drug Control Administration
- Medical Stores
- Colleges and Universities

Objective of Pharmaceutical Chemistry

(i) In pharmaceutical chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. Such a compound could also be called a 'drug'.

(ii) Pharmaceutical chemistry is concerned with the design (drug design) and synthesis of biologically active molecules.

(iii) The aim is to gain new chemical molecules that could enable the discovery of new pharmaceuticals or optimize already known drug structures. Although organic chemistry plays a crucial role and interact with other disciplines, such as molecular biology, structural biology, pharmacology, physical chemistry, biochemistry, pharmacokinetics, pharmaceutical technology, toxicology or with experts from the field of translational medicine, etc.

(iv) To study the relationship between the chemical structure and biological activity of a molecule (structure-activity relationships, SAR) in a quantitative sense (quantitative SAR, QSAR).

(v) Apart from the small synthetic ligands and natural products, pharmaceutical chemistry also focus on the development of modified peptides and proteins, biological agents (e.g. monoclonal antibodies), multifunctional molecular complexes and synthetic vaccines.

Sources and Types of Errors

Error refers to the difference in the standard values and the true value. Errors may be broadly divided into two categories, namely:

1. **Determinate (Systematic) Errors** - These are errors that possess a definite value with a reasonable cause and these avoidable errors may be measured and accounted for rectification. The most important errors belonging to this particular class are:
   - **(a) Personal Errors:** They are exclusively caused due to 'personal equation' of an analyst and do not due to either on the prescribed procedure or methodology involved.
   - **(b) Instrumental Errors:** These are invariably caused due to faulty and uncalibrated instruments, such as: pH meters, UV-spectrophotometers, potentiometers etc.
   - **(c) Reagent Errors:** The errors that are solely introduced by virtue of the individual reagents, for instance, impurities inherently present in reagents; high temperature volatilization of platinum (Pt); unwanted introduction of 'foreign substances' caused by the action of reagents on either porcelain or glass apparatus.
   - **(d) Constant Errors:** They are observed to be rather independent of the magnitude of the measured amount; and turn out to be relatively less significant as the magnitude enhances. Example: error of 0.10 ml is introduced in a series of titrations, hence for a specific titration needing only 10.0 ml of titrant shall represent a relative error of 1% and only 0.2% for a corresponding 50 ml of titrant consumed.
   - **(e) Proportional Errors:** The absolute value of this kind of error changes with the size of the sample in such a fashion that the relative error remains constant. It is usually incorporated by a material that directly interferes in an analytical procedure.
   - **(f) Errors due to Methodology:** Both improper (incorrect) sampling and incompleteness of a reaction often lead to serious errors. A few typical examples invariably encountered in titrimetric and gravimetric analysis.

(g) **Additive Errors:** It has been observed that the additive errors are independent of the quantum of the substances actually present in the assay.

2. **Indeterminate (Random) Errors** - As the name suggests, indeterminate errors cannot be pin-pointed to any specific well-defined reasons. These errors are mostly random in nature and ultimately give rise to high as well as low results with equal probability. They can neither be corrected nor eliminated, and therefore, form the 'ultimate limitation' on the specific measurements.
    1. Repeated measurement of the same variable several times and subsequent refinement to the extent where it is simply a coincidence if the corresponding replicates eventually agree to the last digit.
    2. Both unpredictable and imperceptible factors are unavoidably incorporated in the results what generally appear to be 'random fluctuations' in the measured quantity.
    3. Recognition of specific definite variables which are beyond anyone's control lying very close to the performance limit of an instrument, such as: temperature variations, noise as well as drift from an electronic circuit, and vibrations caused to a building by heavy vehicular-traffic.

Sources of Error

Common sources of error include instrumental, environmental, procedural, handling of equipment and human. All of these errors can be either random or systematic depending on how they affect the results.

Errors are mainly two types random and systematic.

- **Random error** occurs due to chance. There is always some variability when a measurement is made. Random error may be caused by slight fluctuations in an instrument, the environment, or the way a measurement is read, that do not cause the same error every time.
- **Systematic error** gives measurements that are consistently different from the true value in nature, often due to limitations of either the instruments or the procedure. Systematic error is one form of bias. Bias is often caused by instruments that consistently offset the measured value from the true value.
- **Instrumental error** happens when the instruments being used are inaccurate, such as a balance that does not work. A pH meter that reads 0.5 off or a calculator that rounds incorrectly would be sources of instrument error.
- **Environmental error** happens when some factor in the environment, such as an uncommon event, leads to error. For example, if you are trying to measure

the mass of an apple on a scale, and your classroom is windy, the wind may cause the scale to read incorrectly.

- **Procedural error** occurs when different procedures are used to answer the same question and provide slightly different answers. If two people are rounding, and one rounds down and the other rounds up, this is procedural error.
- **Human error** is due to carelessness or to the limitations of human ability. Two types of human error are transcriptional error and estimation error.

Steps to Reduce the Errors

Systematic errors may be reduced substantially and significantly by adopting one of the following procedures rigidly, such as:

(i) Calibration of Instruments, Apparatus and Applying Necessary Corrections: Most of the instruments, commonly used in an analytical laboratory, such as: UV-Spectrophotometer, IR-Spectrophotometer, single pan electric balance, pH-meter, turbidimeter and nephelometer, polarimeter, refractometer and the like must be calibrated duly, before use so as to eliminate any possible errors. In the same manner all apparatus, namely: pipettes, burettes, volumetric flasks, thermometers, weights etc., must be calibrated duly, and the necessary corrections incorporated to the original measurements.

(ii) Performing a Parallel Control Determination: It essentially comprises of performing an altogether separate estimation under almost identical experimental parameters that consists of exactly the same weight of the component as is present in the unknown sample.

(iii) Blank Determination: It may be accomplished by performing a separate parallel estimation, without using the sample at all, and under identical experimental parameters as employed in the actual analysis of the given sample

(iv) Cross-checking Results by Different Methods of Analysis: In certain specific cases the accuracy of a result may be cross-checked by performing another analysis of the same substance by an altogether radically different method.

Accuracy

The accuracy represents the proximity between the standard reference and the observed value during analysis. The ability of the instrument to measure the accurate value is known as accuracy. In other words, the closeness of the measured value to a standard or true value. Accuracy is obtained by taking small readings. The small

reading reduces the error of the calculation. The accuracy of the system is classified into three types as follows:

- **Point Accuracy:** The accuracy of the instrument only at a particular point on its scale is known as point accuracy.
- **Accuracy as Percentage of Scale Range:** The uniform scale range determines the accuracy of a measurement. This can be better understood with the help of the following example: Consider a thermometer having the scale range up to 500°C. The thermometer has an accuracy of ±0.5, i.e. ±0.5 percent of increase or decrease in the value of the instrument is negligible. But if the reading is more or less than 0.5°C, it is considered a high-value error.
- **Accuracy as Percentage of True Value:** Such type of accuracy of the instruments is determined by identifying the measured value regarding their true value. The accuracy of the instruments is neglected up to ± 0.5 percent from the true value.

Precision

The precision is the closeness of results obtained from analysis of the same sample repetitively.

The closeness of two or more measurements to each other is known as the precision of a substance. If you weigh a given substance five times and get 3.2 kg each time, then your measurement is very precise but not necessarily accurate. Precision is independent of accuracy. The below example will tell you about how you can be precise but not accurate and vice versa. Precision is sometimes separated into:

- **Repeatability:** The variation arising when the conditions are kept identical and repeated measurements are taken during a short time period.
- **Reproducibility:** The variation arises using the same measurement process among different instruments and operators, and over longer time periods.

Significant Figures

Significant figures (also known as the significant digits, precision or resolution) of a number in positional notation are digits in the number that are reliable and absolutely necessary to indicate the quantity of something.

If a number expressing the result of measurement of something (e.g., length, pressure, volume, or mass) has more digits than the digits allowed by the measurement resolution, only the digits allowed by the measurement resolution are reliable so only these can be significant figures.

Of the significant figures in a number, the most significant is the digit with the highest exponent value (simply the left-most significant figure), and the least

significant is the digit with the lowest exponent value (simply the right-most significant figure).

Impurities in Pharmaceuticals

Impurity is a substance which is not part of the drug or medicinal substance. It is foreign substance present in the formulation other than the drug. Chemical purity means freedom from foreign matter (Impurity). The substance used in pharmaceutical field should be almost pure so that they can be used safely. It is rather difficult to obtain an almost pure substance. We find substances and chemicals with varying degree of purity because the purity of substance depends upon several factors such as their method of manufacture, type of recrystallisation or purification process.

In the pharmaceutical field, one deals with a large number of drugs, chemicals or other substance which are used in formulations. All such materials need to be pure. However, it is almost impossible to get an absolutely pure material as impurities gets incorporated into them either during manufacture, purification or storage.

Test for purity: The pharmacopoeias prescribe test for purity for substance in order to ensure their reasonable freedom from the undesirable impurities. Test for purity is in fact tests for detecting the presence of impurities and they fix the limits of tolerance for these impurities. The test for purity does not have the aim of ensuring freedom of substance from every possible impurity.

The following certain tests are carried out on the substances.

(i) **Odour and Colour:** These test are employed only when other test for purity are not applicable. Therefore, these tests have limited importance. These tests are valuable to know whether the substance is reasonably aesthetic and hygienic or not.

(ii) **Physical Constants:** Melting point, boiling point, refractive index. Optical rotation is the reliable physical constants. The determination of physical constants ensure whether the substance are reasonably free from other substances. This test fails to indicate the nature of the impurity.

(iii) **Humidity/Moisture Content:** The amount of moisture content in medicinal substance is determined to estimate the content of water of recrystallisation if present in the compound. It give idea about storage condition of certain drugs like ergot, digitalis.

(iv) **Insoluble Constituents:** The compound which is soluble in water gives a turbid solution if soluble matter is present. The turbidity is due to insoluble material or constituents present in the watersoluble compound. The measurement of turbidity or opalescence helps to indicate the extent of insoluble constituents present as an impurity in the compound.

(v) **Organic impurities:** These may be from raw material or intermediate products or by products in reaction. Therefore, for some of these objectionable organic impurities the tests are prescribed in official books.

(vi) **Acidity and Alkalinity:** Excess of acidity and alkalinity has effect on keeping qualities of the compounds as well as the compounds with which they may be mixed.

(vii) **Anions:** Acids like H_2SO_4 and HCl are widely used in the manufacture of medicinal substances. Therefore, the chloride and sulphate ions are commonly present as impurity in many of the medicinal substance. Hence test for anions like Cl^-, $SO4^{2-}$ is prescribed in official books.

(viii) **Cations:** Tests for cation include for sodium, potassium ammonium radical and for heavy metal like iron, lead, copper etc. These impurities are toxic in nature and controlled by performing the limit test for Lead and Arsenic.

(ix) **Ash:** Residue remaining after incineration is the ash content of the drugs, which represents the inorganic salts naturally occuring in the drugs. Determination of ash value is performed to have the idea about content of foreign cations and heavy metals. In organic compounds, the alkali salts are generally present as impurities. In such a case the determination of ash value is preferable. It is also useful to judge the identity or purity of crude drugs.

(x) **Loss on Drying (LoD):** In this test, absorbed water or water of hydration is determined by drying under specified conditions. Loss in weight due to drying also represents the residual volatile constituents including organic solvents as well as water.

(xi) **Loss on Ignition (LoI):** This type of test is applied to stable substances which are liable to contain thermolabile impurities. This is applied to two classes of substances,

- Those which are completely volatile when ignited and
- Those which undergo a major decomposition leaving a resistance of definite composition.

Effect of impurities: The impurities present in the pharmaceutical substance may,

- Have toxic effect if present beyond the limits.
- Change the physical and chemical properties of the drug making it unsuitable for medicinal use.
- Be incompatible with other substance.
- Lower the shelf life of the substance.
- Cause technical difficulties in the formulation.
- Cause change in colour, taste, odour etc. making the substance unhygienic.

Types of impurities: Following types of impurities are commonly present in the pharmaceutical substance or preparations.

(i) The impurities which produce toxic effect on body, if present beyond the prescribed limit e.g. Lead and Arsenic impurities.

(ii) Impurities which are harmless but if present beyond the limit in pharmaceutical substances, lower the active strength of that substance e.g. Impurities of sodium salts in potassium salts.

(iii) Impurities which, if present beyond the limit affect the storage property of the pharmaceuticals e.g. presence of moisture beyond the limit, may loose the free flowing property of substance or may decompose the substance.

(iv) Impurities causing technical difficulties while using the substance in which it is present e.g. presence of carbonate impurity in ammonia solution.

(v) Impurities such as taste, odour, colour or appearance which are easily detectable by the senses and make the substance unaesthetic or unhygienic. e.g. phenolic impurities present in sodium salicylate alters its odour.

Traces of magnesium salts in sodium chloride renders it damp and changing its appearance.

Factors to be considered while fixing the limit of impurities:

(i) Use of the substance for which the limit of impurities is to be fixed.

(ii) Minimum quantities of impurities likely to be harmful or to cause undesirable results in dispensing in keeping qualities.

(iii) Practicability of getting the particular limit or particular standard of quality.

(iv) Harmfulness of impurity.

Sources of Impurities

1. **Raw Materials:** If the impurities are present in the raw material, it may come in the final product through the manufacturing processes. e.g. Sodium chloride prepared from rock salt contains traces of calcium and magnesium compounds & Zinc oxide prepared from zinc metal may contain traces of copper, magnesium, nickel, iron and arsenic.
2. **Methods used in manufacture:** There are different number of methods available to manufacture. Some impurities may come into final product during manufacturing processes. So, to avoid impurities, suitable manufacturing process should be adopted.
3. **Intermediate Product:** The intermediate product may come along the process in the final product as impurities. e.g. In the preparation of potassium iodide from potassium hydroxide and iodine, potassium iodate is an intermediate product which is sometimes found in the final product.

4. **Material of the plant:** The vessels used in the manufacturing process are generally made up of metals like iron, copper, zinc, nickel, aluminium, steel etc. Due to solvent action on the material of the plant the traces of metals as a impurities may come in the product. For example, water pipe may contain lead which may accompany the final product.
5. **Impurities in atmosphere:** The atmospheric contaminants like dust, arsenic, carbon dioxide, water vapours etc. may contaminate the substances which are affected by their action.
6. **Adulteration:** Some pharmaceutical products may be adulterated with cheaper substances. e.g. Potassium bromide may be adulterated with sodium bromide. Therefore, it is advisable to purchase drugs from reputed manufacturer.
7. **Defective storage of final products (Adequate storage):** If there is improper storage of pharmaceutical products, same may undergo chemical decomposition. e.g. Iodine react with cork, rubber and some metals therefore it should be stored in glass bottles fitted with glass stoppers & Potassium hydroxide absorbs CO_2 on exposure to air and has effect on lead glass. Therefore, it should be stored in stoppered green glass bottle which is lead free.
8. **Solvent:** Water is mainly used as a solvent in various pharmaceutical product. Water contains various ions like calcium, magnesium, chloride etc. These impurities come along with water in the final product.
9. **Reagents used in the manufacturing:** If the reagents are impure, the impurity may come in the final product. If sulphuric acid is prepared by lead chamber process it contains traces of lead which may come in the final product as an impurity.
10. **Reagents used to remove impurities:** Potassium bromide is used to remove excess of sulphate, but potassium bromide may contain traces of barium which may contaminate the product.

Limit Test

Limit test are quantitative or semi-quantitative tests designed to identify and control small quantities of impurities which are likely to be present in pharmaceutical substance. These limit tests involve simple comparison of opalescence, turbidity or colour with standards prescribed in pharmacopoeias. The standard for opalescence, colour, turbidity is fixed.

In the limit tests, the extent of turbidity, opalescence or colour produced is influenced by the presence of other impurities present in the substance and also by the variations in time and method of performance of the tests and hence, the pharmacopoeias don't prescribe numerical values for the limit tests.

Thus, the limit tests are performed to know whether the impurities in the substance are below the limit or beyond the limit.

Tolerable Limit: It is the value upto which the impurity is accepted and is permissible in the pharmaceutical preparation or substance.

Limit Test for Chloride

Principle: It is based on the reaction between silver nitrate and soluble chloride resulting in formation of opalescence of silver chloride insoluble in dilute nitric acid.

$$Cl + AgNO_3 \xrightarrow{\text{dil. } HNO_3} AgCl \downarrow + NO_3$$

The extent of opalescence formed depends upon the amount of silver chloride formed and therefore, on the amount of chloride impurity present in the substance under test. The opalescence produced by a given amount of the substance is compared with the standard opalescence produced by adding silver nitrate into a standard solution. If the opalescence from the sample is less than the standard opalescence the sample passes the limit test and vice versa.

The principle according to I.P. 1985 is similar to that I.P. 66. But in I.P. 1985, in the preparation of standard opalescence instead of 0.01 N HCl, the use of 0.05845% w/v solution of sodium chloride is recommended.

Procedure

(i) For test solution: Prepare solution of given sample as directed in I.P. and transfer it in Nessler's cylinder. Add to it 10 ml of dilute HNO_3. Dilute to 50 ml with water. Add 1 ml of 5% silver nitrate solution and stir immediately and allow to stand for five minutes.

(ii) For standard solution: Take 1ml of 0.05845% w/v solution of sodium chloride by pipette in Nessler's cylinder. Add 10 ml of dilute Nitric acid in it. Dilute to 50 ml with water. Add 1ml of 5% silver nitrate solution. Stir immediately with a glass rod and allow to stand for five minutes.

Procedure for Test solution of Potassium permanganate as per IP 1996:

(a) Weigh accurately 1.5 gm of potassium permanganate and transfer it to 250 ml conical flask.

(b) Add 50 ml of distilled water, heat in water bath.

(c) Add gradually 6ml of 95% ethanol and cool.

(d) Dilute to 60 ml with distilled water and filter, the filtrate is colourless.

(e) Take 40 ml of above filtrate in a labelled Nessler's Cylinder (T).

(f) Add 10 ml of dilute HNO_3.

(g) Add 1 ml of 0.1 M silver nitrate solution.

(h) Stir immediately with glass rod and allow to stand for 5 minutes, protect from light.
(i) View transversely against a black background.
(j) Compare the opalescence produced with that of standard solution.

Modification Done (For coloured substance) e.g. $KMnO_4$: Since the $KMnO_4$ is violet colour substance. Its original violet colour modifies the opalescence, colour, turbidity produced in the test. Therefore, such compounds are decolorized first and then used in the test. The solution of $KMnO_4$ is first decolorized by boiling with alcohol and filtering the coloured matter, manganese dioxide is produced.

Limit Test for Sulphate

Principle: Limit test for sulphate is based on the reaction between barium chloride and soluble sulphates in the presence of dilute hydrochloric acid.

$$SO_4 + BaCl_2 \xrightarrow{\text{dil. HCl}} BaSO_4 \downarrow + 2Cl^-$$

The turbidity produced by given amount of substance is due to the precipitation of $BaSO_4$ which is compared with that produced standard solution. In I.P. 1985 instead of Barium chloride solution Barium sulphate reagent (B.S.R.) is used.

B.S.R. Contains:

- **Barium Chloride solution:** It act as seeding agent for the precipitation of barium sulphate if the given sample contains sulphate ions.
- **Sulphate free alcohol:** Prevents supersaturation and helps to form uniform turbidity.
- **Potassium Sulphate:** Increases the sensitivity of the test.

The ionic concentration of BSR is adjusted such that the solubility products of barium sulphate exceed. In sulphate limit test I.P. 1985, in the preparation of standard turbidity instead of 0.01N, H_2SO_4, the use of 0.1089% w/v solution of potassium sulphate is recommended.

Procedure (I.P. 1985):

Test Solution: Prepare solution of the given substance as directed in I.P. 85 and transfer it in Nessler's cylinder. Add to it 2 ml of dil. HCl. Dilute to 45 ml with water and add 5 ml of barium sulphate reagent (B.S.R.). Stir immediately and allow to stand for five minutes.

Standard Solution: Take 1ml of 0.1089 % w/v solution of potassium sulphate in Nessler's cylinder. Add to it 2 ml dilute hydrochloric acid. Dilute it to 45 ml with water. Add 5 ml of Barium Sulphate reagent. Stir immediately and allow to stand for

five minutes. Compare turbidity produced by test solution with that of standard solution.

Procedure for Sulphate limit test I.P. 96: To 1.0 ml of a 2.5% w/v solution of barium chloride in Nessler's 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 the solution prepared as directed in the individual monograph or a solution of specified quantity of the substance being examined in 15 ml of water and 0.15 ml of 5M acetic acid. Add sufficient water to produce 50 ml, stir immediately with glass rod and allow to stand for 5 minutes. When viewed transversely against black background only, opalescence produced is more intense than that obtained by treating in the same manner 15 ml of sulphate standard solution (10 ppm SO_4) in place of solution being examined.

Note: The solution used for this test should be prepared with distilled water.

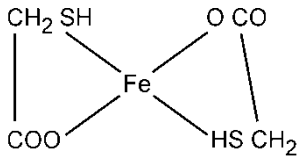
Limit Test of Iron

Principle: It is based upon the reaction of iron with thioglycollic acid, in ammonical solution in presence of citric acid, to produce pale pink to deep reddish purple colour. The colour produced is due to formation of ferrous compound, ferrous thioglycollate which is stable in absence of air but fades in air due to oxidation. The purple colour is developed only in alkaline medium so ammonia solutions are used. But ammonia reacts with iron forming precipitate, citric acid prevents precipitate of iron with ammonia by forming a complex with it.

Fe^{3+} (ferric) form is reduced to (ferrous) Fe^{2+}.

$$2HSCH_2COOH + Fe^{2+} \longrightarrow Fe(SHCH_2COO)^{-}{}_2 + 2H^+$$

OR



(Ferrous thioglycollate complex)

Preparation of standard solution of Iron:

As per I.P. 1996: (Iron standard solution 20 ppm Fe)

Dilute 1 volume of a 0.1726 % w/v solution of ferric ammonium sulphate in 0.05m sulphuric acid to 10 volumes with water contain iron in ferric state.

Procedure:

Test solution: Dissolve the specified weight of substance in 40 ml water or prepare a solution as per I.P. Add 2 ml of 20% w/v solution of Iron free citric acid in water and

2 drops of thioglycollic acid, mix, make alkaline with iron free solution of ammonia, dilute to 50 ml with water and allow to stand for 5 minutes.

Standard solution: Dilute 2 ml of standard solution of iron with 40 ml of water, add 2 ml of 20% w/v solution of iron free citric acid in water and drops of thioglycollic acid, mix make it alkaline with iron free solution of ammonia, dilute to 20 ml with water and allow to stand for five minutes. If the colour produced by test solution is less than that of standard, the sample passes the limit test for iron and vice versa.

Limit Test for Heavy Metal: The I.P. 1985 describes three methods for limit test for Heavy metals.

Method A:

For colourless substance:

Principle: The test is based on the reaction between hydrogen sulphide and certain heavy metals (such as lead, copper, nickel, cobalt, bismuth) leading to the formation of sulphides of respective metals in the presence of dilute acetic acid.

$$\text{Heavy Metals} + H_2S \xrightarrow[\text{Acid}]{\text{Acetic}} \text{Sulphides of Heavy metal} + 2H^+$$

Acetic acid is added to maintain pH 3 to 4. Therefore the sulphide formed are distributed in colloidal state and produce brownish coloured solution.

Procedure:

Test: Place 25 ml of solution prepared according to I.P. in 50 ml Nessler's cylinders. Adjust with dilute acetic acid or ammonia to a pH between 3 to 4, dilute with distilled water to 35 ml and mix. Add 10 ml of freshly prepared saturated solution of hydrogen sulphide. Dilute to 50 ml with distilled water. Stir with glass rod and allow it to stand for 5 min.

Standard: Place 2 ml of standard lead solution in a 50 ml Nessler's cylinder and dilute to 25 ml with distilled water. Adjust with dilute acetic acid or ammonia to a pH between 3-4. Dilute with distilled water to 35 ml and mix.

Add 10 ml of freshly prepared saturated solution of hydrogen sulphide. Dilute to 50 ml with distilled water. Stir with glass rod and allow it to stand for 5 min.

Compare the colour of sample and standard.

Method B: For coloured substance:

It is similar to method A, only difference is test (sample) is given special treatment (with sulphuric acid, ignition nitric acid, ignition HCl and finally digestion with water etc.) to make it colourless before preparing its solution.

Method C: Used for substance which forms clear colourless solution with NaOH.

Principle: It is based on the reaction of heavy metals with sodium sulphide in an alkaline medium leading to formation of heavy metal sulphides.

$$\text{Heavy metals} + Na_2S \xrightarrow[\text{medium}]{\text{alkaline}} \text{Sulphide of heavy metals}$$

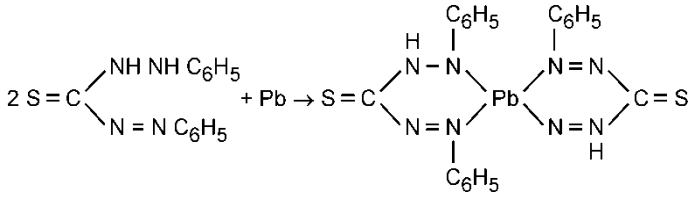
Procedure:

Test: Take 25 ml sample solution prepared as per IP in Nessler's cylinder. Add 5 ml of dilute NaOH solution. Dilute to 50 ml with distilled water. Add 5 drops of sodium sulphide solution.

Stir with glass rod and allow it to stand for 5 min.

Standard: Take 2 ml of standard lead solution in a Nessler's cylinder and dilute it with distilled water to 25 ml. Add 5 ml of dilute sodium hydroxide solution. Dilute to 50 ml with distilled water. Add 5 drops of sodium sulphide solution. Stir with glass rod and allow it to stand for 5 minute. Compare the colour of sample and standard.

Limit test for Lead: As per I.P. and USP it, is based upon the reaction between lead and diphenylthio-carbazone (Dithizone). Dithizone in chloroform, extracts lead from alkaline aqueous solution and lead Dithizone complex. (Red Colour).



The original dithizone has green colour in chloroform thus the lead-dithizone shows a violet colour. The intensity of colour of complex depends upon the amount of lead in solution.The colour of lead dithizone complex in chloroform is compared with a standard volume of lead solution, treated in same manner.

Importance of Potassium Cyanide: The interference and influence of other metal ions etc. is eliminated by adjusting the optimum pH for the extraction by using ammonium nitrate, potassium cyanide, hydroxylamine hydrochloride reagents etc.

P.P.M. (Parts Per Million): It may be defined as the number of parts by weight of a impurity present in one million parts by weight of substance under test.

Limit Test for Arsenic:

Principle and Reactions: It is based upon the conversion of arsenic if present in the sample to the arsine gas with the help of reducing agents. First of all the arsenic present is converted to Arsenic acid in acidic medium.

$$As^{5+} \xrightarrow[\text{medium}]{\text{Acidic}} \underset{\text{Arsenic acid}}{H_3As\,O_4}$$

The arsenic acid is then reduced to Arsenious acid with the help of reducing agents.

$$H_3AsO_4 \xrightarrow[KI]{SnCl_2} \underset{\text{Arsenious Acid}}{H_3\,As\,O_3}$$

The arsenious acid is further reduced to arsine gas by Nascent hydrogen (which is produced by zinc and hydrochloric acid)

$$H_3AsO_3 + \underset{\substack{\text{Nascent}\\\text{Hydrogen}}}{6[H]} \xrightarrow[HCl]{Zn} \underset{\text{Arsine gas}}{AsH_3} + 3H_2$$

The arsine gas produced reacts with mercuric chloride paper to produce a yellow stain of mercuric arsenide.

$$\underset{\substack{\text{Arsine}\\\text{gas}}}{2\,AsH_3} + \underset{\substack{\text{Mercuric}\\\text{chloride}}}{HgCl_2} \longrightarrow Hg\begin{matrix}\diagup AsH_2\\ \\ \diagdown AsH_2\end{matrix} + 2\,HCl$$

Mercuric arsenide

The stain produced by sample is compared with stain produced by standard.

Use of Reagents:

- HCl and Zn produce Nascent hydrogens.
- Potassium iodide reduces pentavalent arsenic to trivalent.
- Stannous chloride gives complete evolution of Arsine gas.
- Lead acetate cotton plug prevent the formation of black stain to mercuric chloride paper produced by sulphide impurities which are present in zinc.
- It also trap the impurities evolved along with Arsine gas.
- Granulated zinc helps in steady and prolong evolution of nascent hydrogen.

Procedure:

- Test (sample): Take 50 ml of distilled water in the bottle of arsenic test apparatus.
- Add 2.5 gm of ammonium chloride sample to this.
- Add 10 ml of stannated HCl acid.
- Add 1 gm of stannated KI.
- Add 10 mg of granulated zinc.
- Allow the reaction to proceed for 40 minutes.

Standard:

- Take 50 ml of distilled water in the bottle of another arsenic test apparatus.
- Add 1 ml of dilute arsenic solution to this.
- Add 10 ml stannated HCl acid.
- Add 1 gm of KI.
- Add 10 mg of granulated zinc.
- Allow the reaction to proceed for 40 minutes.
- The apparatus used to perform the limit test of Arsenic is called as GUTZEIT Apparatus.
- It consist of wide mouth glass bottle having capacity of 120 ml and mouth diameter 2.5 cm.
- The mouth of bottle is fitted with rubber bung through which passes a glass tube of length 200 mm, having internal diameter 6.5 mm and outer diameter 8 mm. The lower end of tube is constricted to about 1mm and have a hole not less than 2 mm in diameter to provide alternate passage for arsine gas.
- The other end of glass tube is cut smooth and carries rubber bungs. (25 × 25mm).

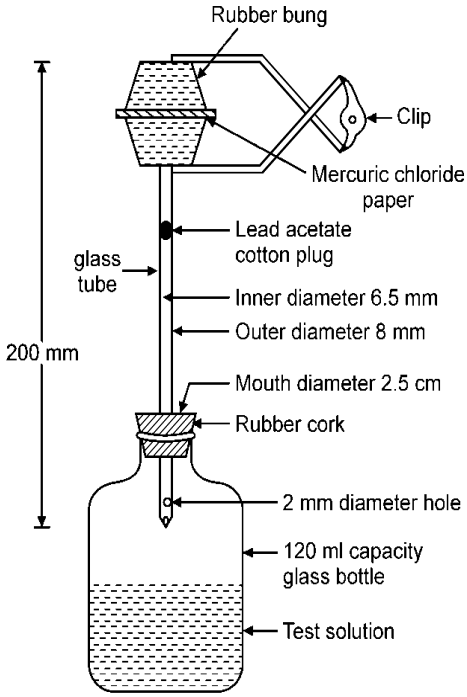


Fig. 1.1 GUTZEIT apparatus

- Mercuric chloride paper is sandwitched between the rubber bungs. The rubber bungs are held in place by means of clip.
- The borings of the two bungs meet to form a true tube of 6.5 mm diameter by a diaphragm of mercuric chloride paper.
- A cotton wool moistened with lead acetate solution and dried, is kept in glass tube.
- When the reaction starts, the arsine gas is formed which goes upward through glass tube. The impurities present with arsine gas are trapped by lead acetate cotton plug. The arsine gas passes through lead acetate cotton plug and reacts with mercuric chloride paper to form yellow stain.

Points to Remember

- Error refers to the difference in the standard values and the true value.
- Common sources of error include instrumental, environmental, procedural, and human.
- The accuracy represents the proximity between the standard reference and the observed value during analysis.
- The precision is the closeness of results obtained from analysis of the same sample repetitively.
- Impurity is a substance which is not part of the drug or medicinal substance. It is foreign substance present in the formulation other than the drug.
- Limit test are quantitative or semi-quantitative tests designed to identify and control small quantities of impurities which are likely to be present in pharmaceutical substance.
- Limit test of chloride is based on the reaction between silver nitrate and soluble chloride resulting in formation of opalescence of silver chloride insoluble in dilute nitric acid.
- Limit test for sulphate is based on the reaction between barium chloride and soluble sulphates in the presence of dilute hydrochloric acid.
- Limit test for iron is based upon the reaction of iron with thioglycollic acid, in ammonical solution in presence of citric acid, to produce pale pink to deep reddish purple colour.
- Limit test for heavy metals is based on the reaction between hydrogen sulphide and certain heavy metals (such as lead, copper, nickel, cobalt, bismuth) leading to the formation of sulphides of respective metals in the presence of dilute acetic acid.
- Limit test for lead is based upon the reaction between lead and diphenylthiocarbazone (Dithizone). Dithizone in chloroform, extracts lead from alkaline aqueous solution and lead Dithizone complex.

- Limit test for arsenic based upon the conversion of arsenic if present in the sample to the arsine gas with the help of reducing agents.
- Pharmaceutical Chemistry is the most important branch of Pharmaceutical Sciences. It includes everything about the processes of drug development and distribution.

Multiple Choice Questions

1. The limit test for heavy metals does not include the following:
   (A) Lead (C) Nickel
   (B) Copper (D) Selenium
2. Lead discovery is responsible under which sub-area of pharmaceutical chemistry?
   (A) Analytical Chemistry (C) Computational Chemistry
   (B) Organic Chemistry (D) None of these
3. Which is not a source of impurities?
   (A) Finished Goods (C) Intermediate Product
   (B) Raw Mater0069als (D) Methods used in manufacture
4. The limit test for lead is based upon the reaction between lead and which component?
   (A) Silver nitrate (C) Sodium thioglycollate
   (B) Dithizone (D) Arsine
5. Which is not a component in the limit test of arsenic?
   (A) Stannated HCl acid (C) Citric acid
   (B) Stannated KI (D) Granulated zinc
6. Which is not a type of error?
   (A) Instrumental (C) Chemical
   (B) Environmental (D) Human
7. The apparatus used to perform the limit test of Arsenic is called
   (A) Gutzeit Apparatus (C) Soxhelet Apparatus
   (B) Clevenger's Apparatus (D) Franz Apparatus
8. Ferrous thioglycolate complex is formed in which limit test?
   (A) Iron (C) Arsenic
   (B) Lead (D) Chloride

9. Which is not a key subject of pharmaceutical chemistry?
   (A) Analytical Chemistry (C) Organic Chemistry
   (B) Computational Chemistry (D) Explosive Chemistry
10. The area where pharmaceutical chemistry professionals are not have desired scope
   (A) Food industries (C) Health Centers
   (B) Printing (D) Product marketing agencies
11. Impurities in pharmaceutical products come from different sources like
   (A) Raw material (C) Chemical instability
   (B) Manufacturing process (D) All of the above
12. In limit test for sulphates, which acid in diluted form is used?
   (A) Sulfuric acid (C) Nitric acid
   (B) Hydrochloric acid (D) Perchloric acid
13. When iron reacts with thioglycollic acid in the presence of citric acid, what color appears?
   (A) Pale pink to deep reddish purple (C) Blue to Black
   (B) Orange to Red (D) Green to Violet
14. What is the role of citric acid in the limit test of iron?
   (A) Helps enhancing solubility (C) Helps reducing turbidity
   (B) Helps precipitation of iron (D) All of these
15. In limit test for chlorides, which acid in diluted form is used?
   (A) Sulfuric acid (C) Nitric acid
   (B) Hydrochloric acid (D) Perchloric acid
16. Limit tests are performed in
   (A) Flask (C) Measuring cylinder
   (B) Test tube (D) All of these
17. In limit test for heavy metals, which acid in diluted form is used?
   (A) Acetic acid (C) Nitric acid
   (B) Hydrochloric acid (D) Perchloric acid
18. Citric acid forms a soluble complex with iron and prevents its precipitation by ammonia as
   (A) Ferrous hydroxide (C) Ferrous sulphate
   (B) Ferrous chloride (D) Ferrous nitrate

19. In limit test for heavy metals, the reaction between the heavy metals occurs with
    (A) Thioglycollic acid
    (B) Citric acid
    (C) Hydrogen sulphides
    (D) None of them

20. If the opalescence in the sample is less than the standard in limit test for chloride, what does it indicate?
    (A) Sample passed
    (B) Sample failed
    (C) Sample reaction occurs
    (D) No conclusion