PHARMACEUTICAL ANALYSIS:

DEFINITION & SCOPE

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- Introduction
- Definition
- Scope
- Different techniques of Analysis

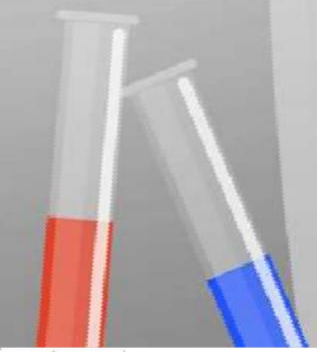
Pharmaceutical analysis or chemical analysis is a technique to identify and/or quantity any sample, substance, compound by using manual, chemical or instrumental qualitative or techniques by quantitative method(s)'.

Analytical chemistry is apprehensive with the theory and practice of techniques used to determine the composition of substance(s). Analytical is very broad and squeezes a wide range of techniques. Chemical analysis is applied in daily life i.e. soaps and detergents act as emulsifiers to surround dirt and grime so can be washed away from clothing, dishes and our bodies.

Analytical chemistry can be Classified into qualitative and quantitative analysis.

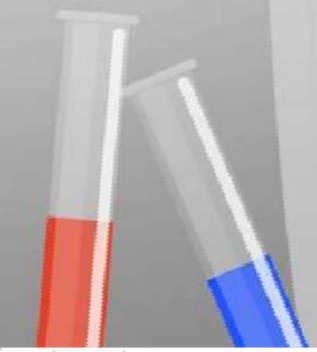
Qualitative analysis is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not.

Quantitative analysis refers to analysis in which the amount or concentration of an substance may be determined and expressed as a numerical value in appropriate units

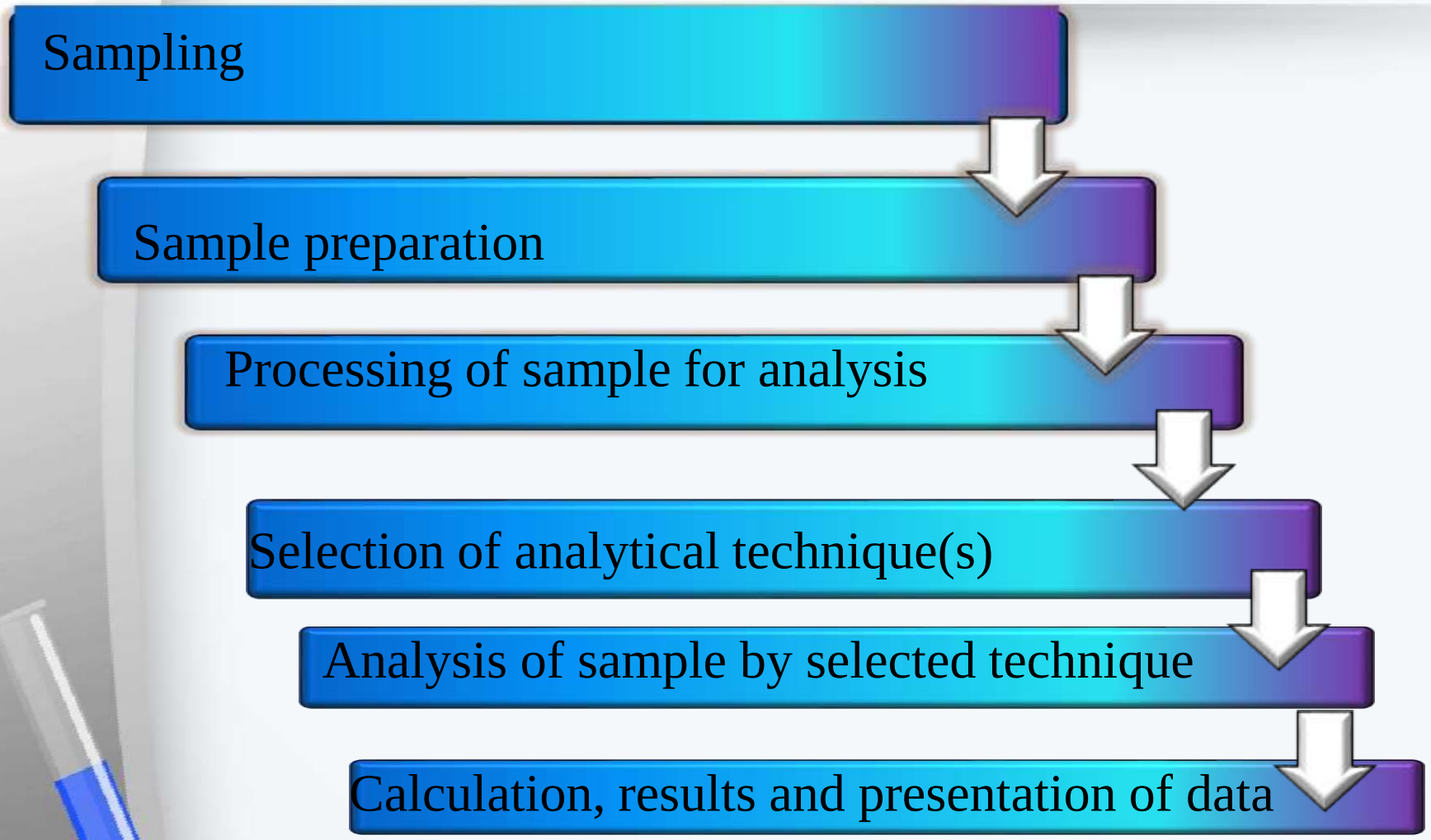


The final mfg. product is subjected to quality control to ensure the claimed quantity of the substance(s)

- Examination of raw materials.
- Development of new products.
- Examination of various drug products.
- Qualitative and quantitative analysis of various samples.
- Diagnosis of disease by various chemical analyses.
- Determination or examination of soil and rock.
- Analysis of different samples of water.
- Determination of radioactive compounds and naturally occurring phytoconstituents.



Steps in Chemical Analysis



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Factors Affecting the Choice of Analytical Technique

Nature of the sample: Acid-base sample requires acid-base titration, oxidizing-reducing sample requires redox titration etc.

Type of the analysis: Volumetric analysis, spectroscopic analysis, chromatographic analysis.

Physical state of the sample: Physical state of the sample plays a major role for selection of the analytical technique i.e. solid, liquid and gaseous samples.

Presence of the impurities: Presence of impurities affects the selection of technique because some analytical methods are not sensitive enough to determination of impurities present in the sample.

Concentration of the analyte in the sample: Selection of the analytical technique is affected by the concentration range of the analyte; some analytical techniques are less sensitive and some are more sensitive. So, more sensitive analytical techniques are selected for the samples containing lower concentration range of the analyte.

Example: pH of the solution can be determined by pH paper and pH meter; but pH meter is highly sensitive and can be differentiating between the two solutions of very low concentration range difference.

Accuracy required: Accuracy of the different analytical techniques varies. For more accurate results highly sensitive techniques are used.

Availability of the instrument: Availability of the instrument(s) for selected analytical technique plays major role. If instrument(s) are not available for the selected technique then the method is useless.

Time required for the analysis: Time required to complete the analysis is thee major factor for selection of the technique. If result is required fast then appropriate method is selected to get the results immediately.

Amount of the sample available: If amount of the sample is not available in sufficient quantity then non-destructive method is preferred (if available).

Cost of the analysis: Selection of the analytical technique is highly governed by the cost of the analysis. Low cost analytical techniques are always preferable, if high cost analytical techniques are not essential.

Classification of Pharmaceutical Analysis Laboratories

1. Government regulatory agencies

Various government regulatory agencies are established by the central and state government; these agencies are continuously monitoring and analysing the various drug samples.

Example: IPC (Indian Pharmacopoeial Commission), CDSCO (Central Drugs Standard Control Organization) etc.

2. Manufacturers of drugs

Large number of drug manufacturing units of government and non-government sectors are equipped with various analytical instruments to analyze the drugs.

3. Manufacturer of raw materials of drugs

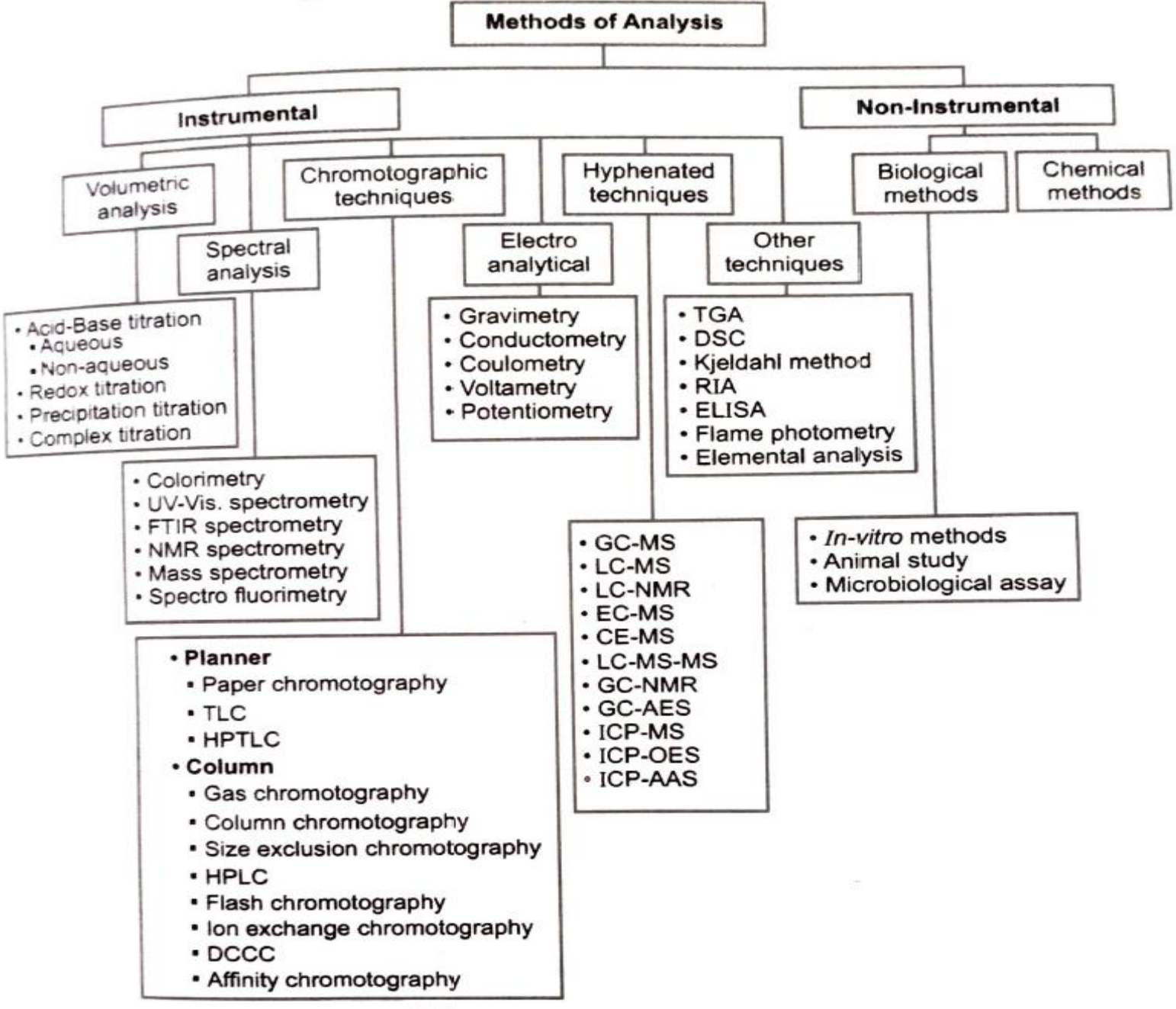
Large number of raw materials of drugs are manufactured by various industries and these raw materials are analysed in the analytical laboratories present in that industry.

4. Universities and non-commercial research centres

Universities, colleges and other non-commercial research centres have their instrumentation laboratory to carry out the analysis of different type of samples.

5. Consulting laboratories

Varieties of consulting laboratories are available to cary out the analysis of the samples by charging on the basis of per sample.


Methods of Analysis
Instrumental
Non-Instrumental
Volumetric analysis
Chromotographic techniques
Hyphenated techniques
Biological methods
Chemical methods
Spectral analysis
Electro analytical
Other techniques
• Acid-Base titration
• Aqueous
• Non-aqueous
• Redox titration
• Precipitation titration
• Complex titration
• Gravimetry
• Conductometry
• Coulometry
• Voltametry
• Potentiometry
• TGA
• DSC
• Kjeldahl method
• RIA
• ELISA
• Flame photometry
• Elemental analysis
• Colorimetry
• UV-Vis. spectrometry
• FTIR spectrometry
• NMR spectrometry
• Mass spectrometry
• Spectro fluorimetry
• GC-MS
• LC-MS
• LC-NMR
• EC-MS
• CE-MS
• LC-MS-MS
• GC-NMR
• GC-AES
• ICP-MS
• ICP-OES
• ICP-AAS
• In-vitro methods
• Animal study
• Microbiological assay
• Planner
• Paper chromotography
• TLC
• HPTLC
• Column
• Gas chromotography
• Column chromotography
• Size exclusion chromotography
• HPLC
• Flash chromotography
• Ion exchange chromotography
• DCCC
• Affinity chromotography

Imp. Question

1. Write a note on definition and scope of Pharmaceutical Analysis

References

1. P, Kumar. A text book of Pharmaceutical Analysis. Nirali Prakashan; 1st Edition, 2017: 1.1-1.4.

DIFFERENT TECHNIQUES OF ANALYSIS

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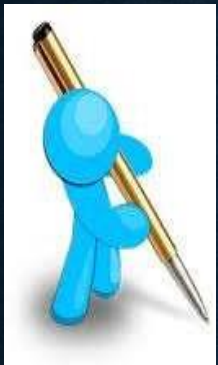

- Defination of Pharmaceutical Analysis
- Different techniques of analysis
- Imp. Question

PHARMACEUTICAL ANALYSIS

Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals

This course has access to the full range of

- Titration method----------}
- Spectroscopic technique/ Spectrophotometry
- Chromatographic method etc.

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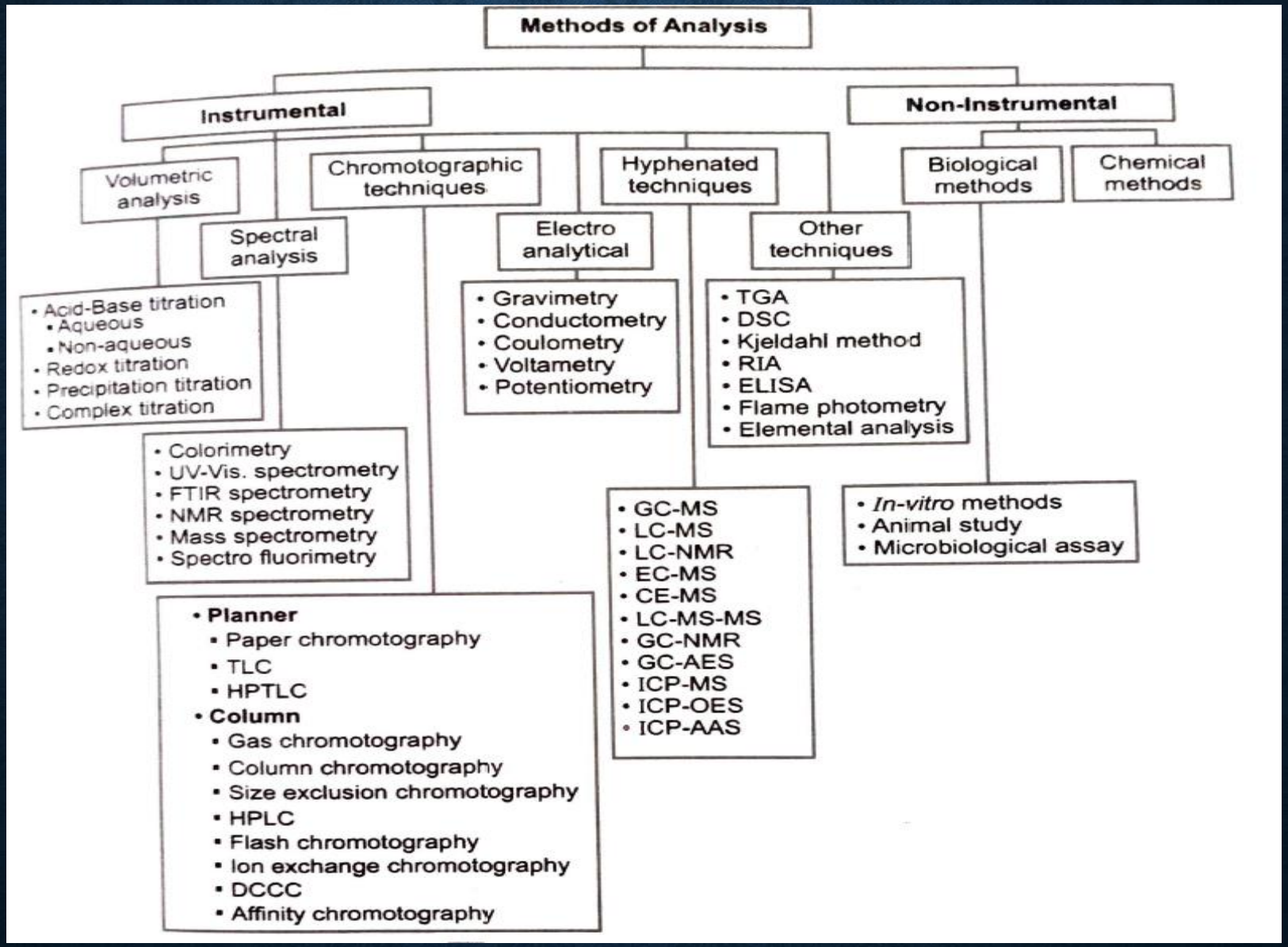
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• UV-Vis. spectrometry
• FTIR spectrometry
• NMR spectrometry
• Mass spectrometry
• Spectro fluorimetry
• GC-MS
• LC-MS
• LC-NMR
• EC-MS
• CE-MS
• LC-MS-MS
• GC-NMR
• GC-AES
• ICP-MS
• ICP-OES
• ICP-AAS
• In-vitro methods
• Animal study
• Microbiological assay
• Planner
• Paper chromotography
• TLC
• HPTLC
• Column
• Gas chromotography
• Column chromotography
• Size exclusion chromotography
• HPLC
• Flash chromotography
• Ion exchange chromotography
• DCCC
• Affinity chromotography

TITRATION METHOD

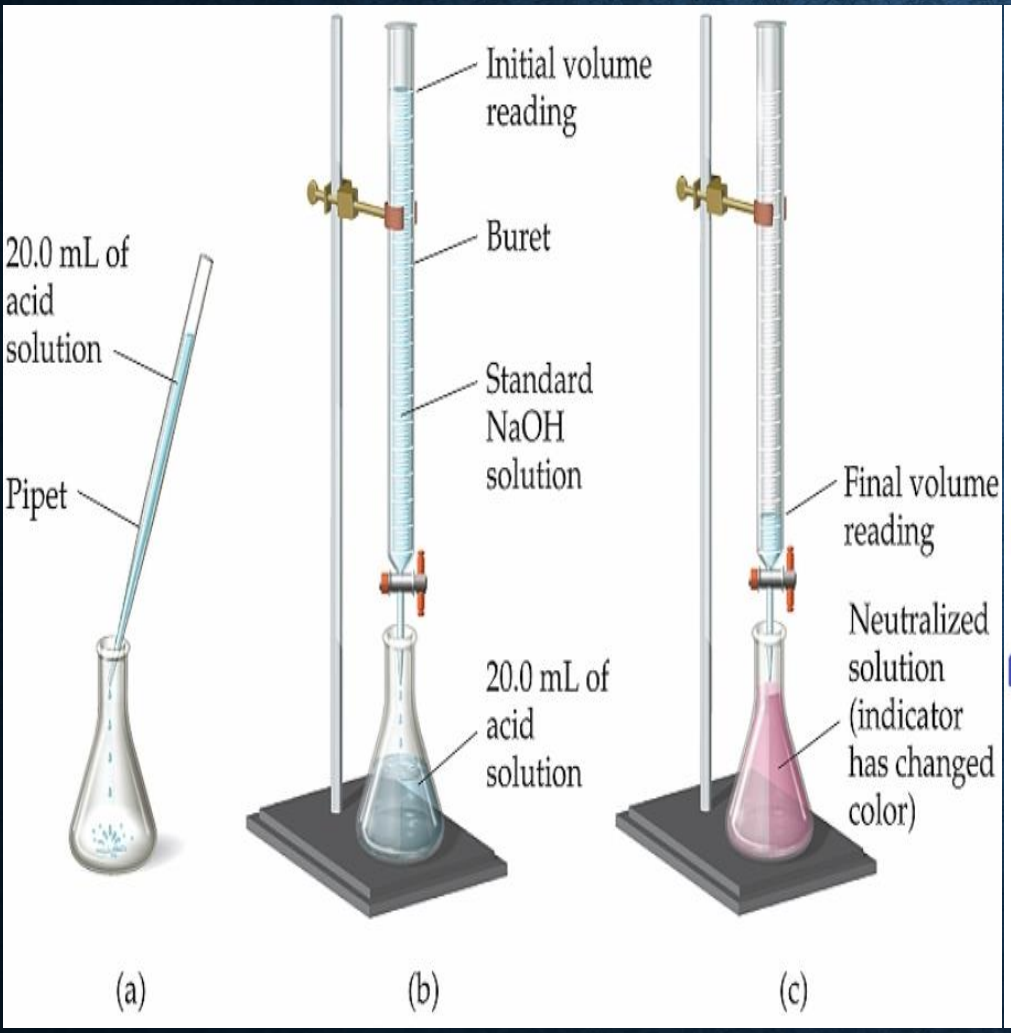
Aqueous acid base titration: An aqueous **acid–base** titration is the determination of the concentration of an acid or base by exactly neutralizing the acid or base with an acid or base of known concentration.

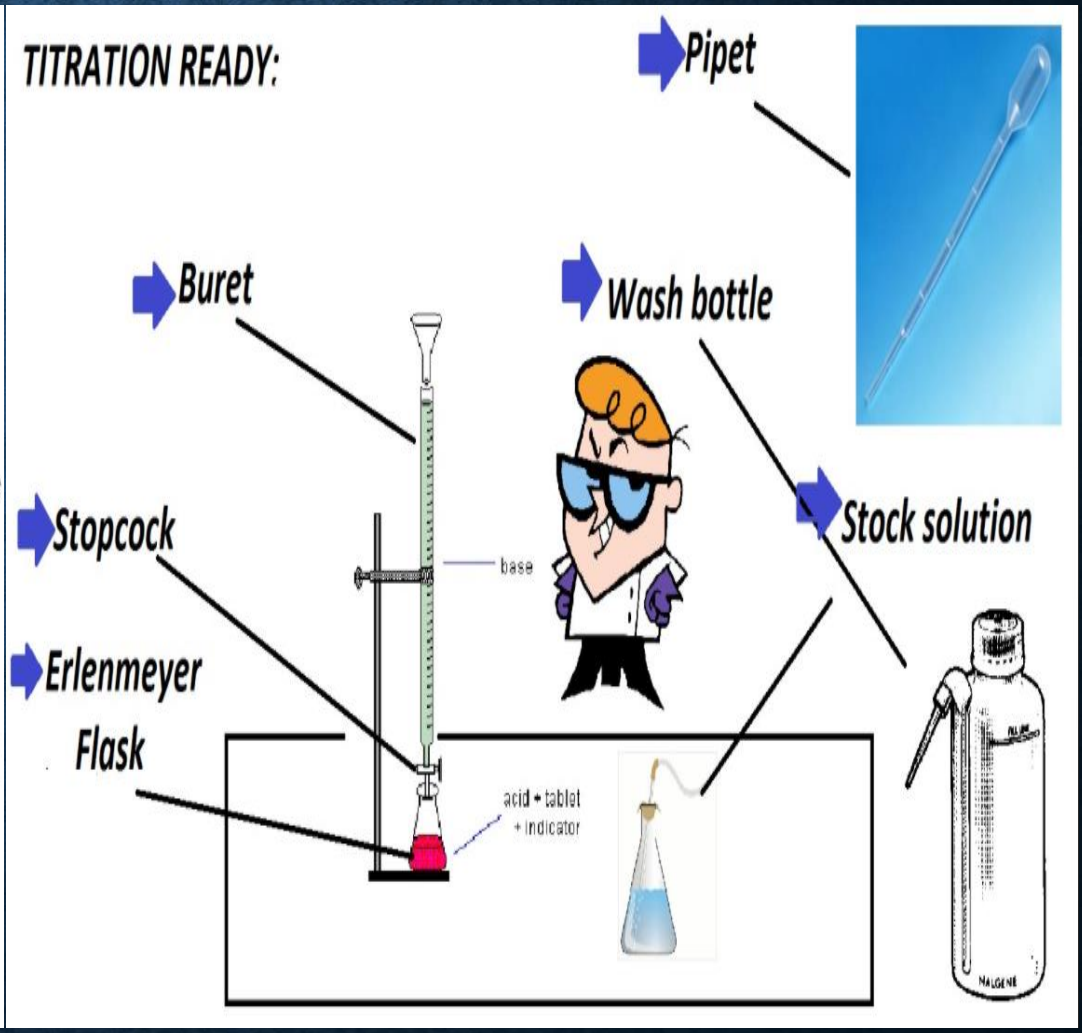
- ✓ This allows for quantitative analysis of the concentration of an unknown acid or base solution.
- ✓ Aqueous acid–base titrations can also be used to find percent purity of chemicals.

Non aqueous acid base titration: Non aqueous titration is the titration of substances dissolved in solvents other than water. it provides a solvent in which organic compounds are soluble.

- ✓ The most commonly used procedure in this titration of organic bases with perchloric acid in *anhydrous* acetic acid.
- ✓ It is the most common titrimetric procedure used in pharmacopoeial assays and is suitable for the titration of very weak acids and very weak bases

TITRATION ASSEMBLY





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TITRATION METHOD

Redox titration: A redox titration is a type of titration based on a redox reaction between the analyte and titrant.

concentration of certain chemicals in pharmaceutical compounds can be determined through redox titration.

Complexometric titration: Complexometric titration is a form of volumetric analysis in which the formation of a colored complex is used to indicate the end point of a titration.

particularly useful for the determination of a mixture of different metal ions in solution

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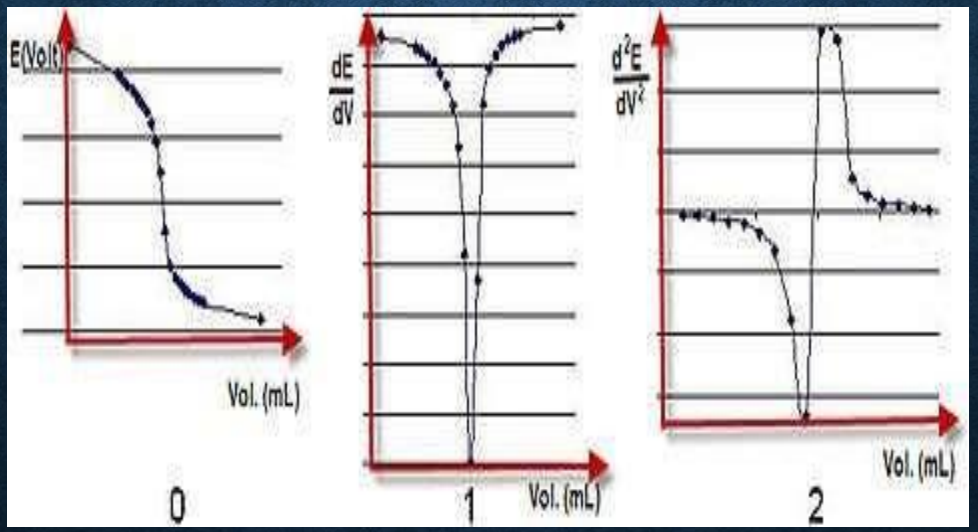
POTENTIOMETRIC TITRATION

- **Potentiometric titration** is a technique similar to direct tritration of a redox reaction. It is a useful means of characterizing an acid. No indicator is used; instead the potential is measured across the analyte, typically an electrolyte solution.



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- ✓ **Amperometric titration:** Refers to a class of titrations in which the equivalence point is determined through measurement of the electric current produced by the titration reaction. It is a form of quantitative analysis.

- ✓ **Aquametry :** Aquametry in analytical chemistry refer to analytical processes to measure the water present in materials.

Use : The methods widely used in aquametry encompasses Karl Fischer titration, distillation, chromatography etc.

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- ✓ **Refractometry** is the method of measuring substances' refractive index (one of their fundamental physical properties) in order to, for example, assess their composition or purity.

- ✓ **Polarimetry** is a sensitive, nondestructive technique for measuring the optical activity exhibited by inorganic and organic compounds.

- ✓ **Nephelometry** is a technique used in immunology to determine the levels of several blood plasma proteins.

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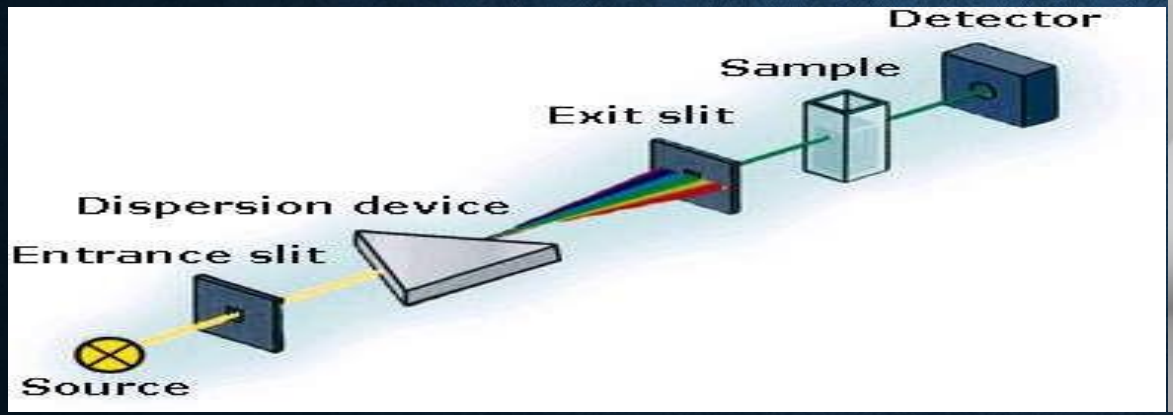
SPECTROSCOPY METHOD

Introduction to Visible & Ultraviolet Spectroscopy:

Spectroscopy is the science which deals with the interaction between a matter (atom/molecule) and an electromagnetic radiation.

Ultraviolet and visible spectroscopy is a type of absorption spectroscopy that uses the ultraviolet and visible parts of the electromagnetic spectrum.



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CHROMATOGRAPHY

Chromatographic Methods:

Chromatography is usually a technique for separating and / or identifying the components in a mixture. It is powerful method in industry.

Some major types of chromatography:

- Paper chromatography
- Gas chromatography
- Liquid chromatography
- High performance liquid chromatography
- Gel filtration chromatography

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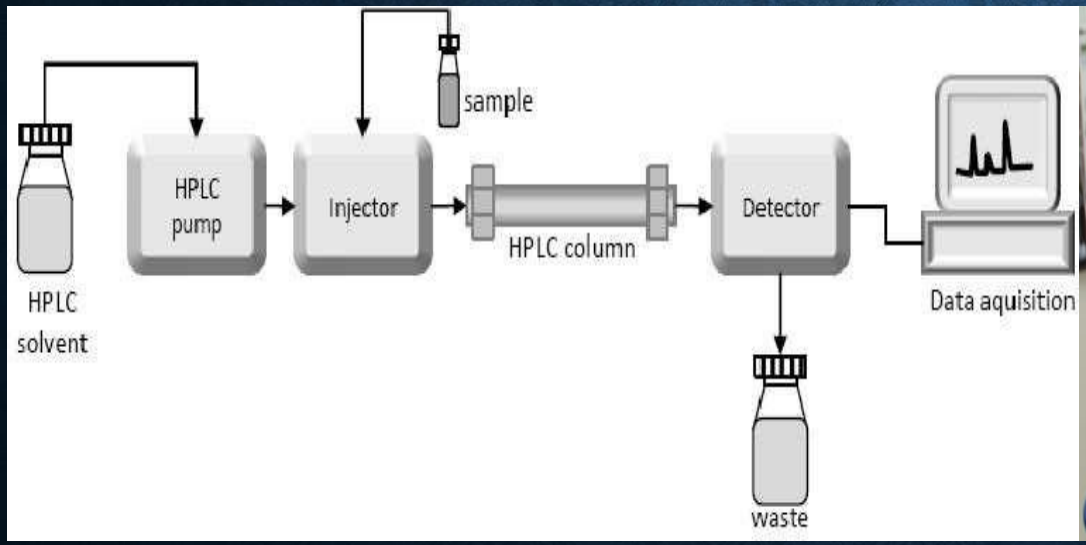
HPLC

High Performance Liquid Chromatography:

High performance liquid chromatography (HPLC) is a very efficient separation technique, that is, it yields excellent separation in a very short period of time.

HPLC is a form of column chromatography. Its frequently used in biochemistry and analytical chemistry to separate component mixture.



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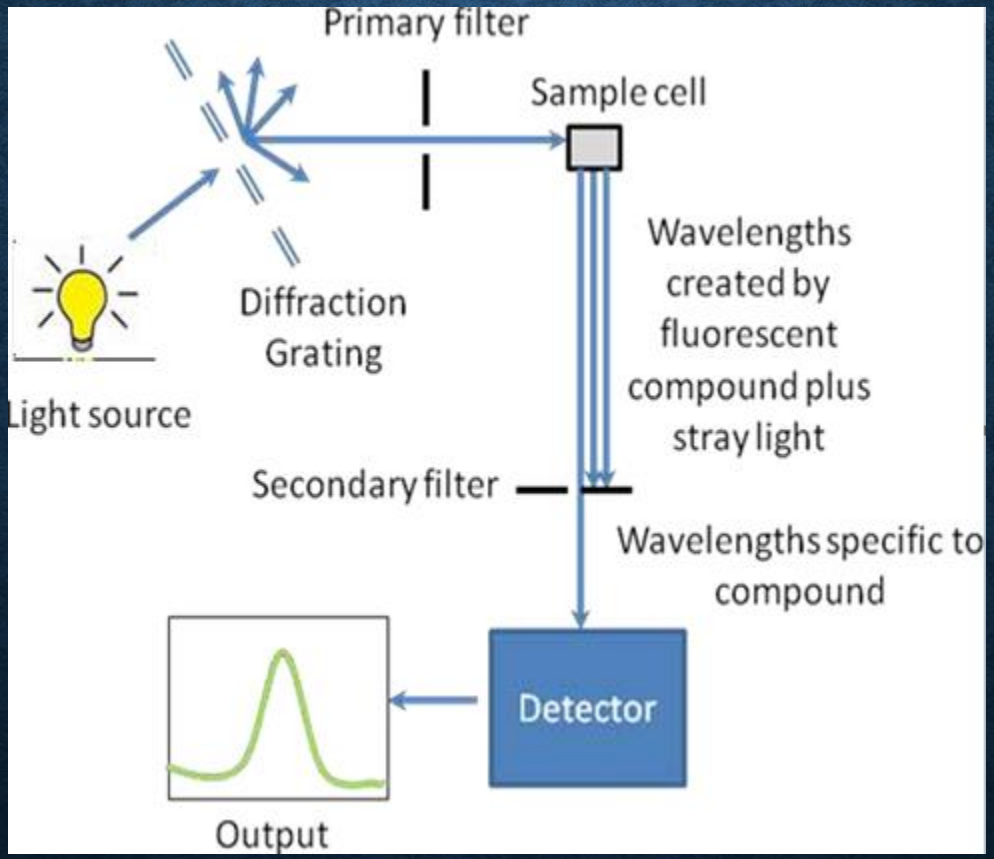
Flurometry:

An analytic method for detecting fluorescent compound using a beam of ultraviolet light that excites the compounds and causes them to emit visible light.



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Good Manufacturing Practices:

GMP is a part of a quality system covering the manufacture and testing of pharmaceutical dosage forms or drugs.GMP are guidance that can impact the quality of product.

Quality Assurance:

QA is a wide-ranging concept,which covers all matters,which individually or collectively influence the quality of a product

Quality Control:

QC is the part of good manufacturing process which is concerned with sampling,specification and testing and with the organisation.

Hazards associated with chemicals and laboratory safety

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Calibration:

Calibration is a process by which ensure that an instrument readings are accurate with reference to established standards.

Calibration is performed using primary reference standards.

Validation:

Validation is the action of checking or proving the validity or accuracy of something in pharmaceutical analysis.

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UV-VISIBLE,INFRARED SPECTROSCOPY:

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet, visible or, infrared spectral region. . In these region of the electromagnetic spectrum, sample atoms and molecules undergo electronic transitions (from ground state to excited) by absorbing radiation energy from a light source.

USE : Used in qualitative and quantitative analysis, sample identification.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is around (60–1000 MHz).

USE: Study and research purposes, molecular physics, structure, crystal and non crystalline material analysis etc.

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MASS SPECTROSCOPY:

In this spectroscopy sample molecules are bombarded with an energetic beam of electrons. Molecules are then ionised and fragmented. Each kind of ion has a particular ratio of mass to charge i.e. (m/e ratio). For most ions the charge is one and thus m/e ratio is simply the molecular mass of the ion.

USE: Isotope dating, protein characterization, glycan analysis, space exploration etc.

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ATOMIC ABSORPTION SPECTROSCOPY

- ✓ Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state.

- ✓ Uses in different areas of chemistry such as clinical analysis of metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue.

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ANALYTICAL TOOLS

Study of other analytical tools include:

→Differential scanning calorimetry (DSC)
→Differential thermal analysis (DTA)
→Near infrared detectors(NIR)

These all are thermo analytical methods.

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IMPORTANCE OF PHARMACEUTICAL ANALYSIS

- Identity of the drug in the formulated product.
- Determination of active ingredient or additional impurities.
- Stability of the drug.
- Rate of drug from its formulation.
- Identity and purity of pure drug that meet specification.
- Concentrations of specified impurities.
- Concentrations of drug in plasma or biological fluids.
- Determine pk_a values, partition coefficients , solubilities, and stability of drug under development.

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IMP. QUESTION

1. Define Pharmaceutical Analysis and Describe different techniques involved in Analysis.

OR

Write a brief note on different techniques of analysis.

THANK YOU

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METHODS OF EXPRESSING CONCENTRATION

1. Concentration in Parts Per Million (ppm)

The parts of a component per million parts (10^6) of the solution.

ppm(A)=Mass of A Total mass of the solution×106

2. Mass Percentage (w/w):

When the concentration is expressed as the percent of one component in the solution by mass it is called mass percentage (w/w). Suppose we have a solution containing component A as the solute and B as the solvent, then its mass percentage is expressed as:

Mass % of A = Mass of component A in the solutionTotal mass of the solution×100

3. Volume Percentage (V/V):

Sometimes we express the concentration as a percent of one component in the solution by volume, it is then called as volume percentage and is given as:

volume % of A = Volume of component A in the solutionTotal volume of the solution×100
For example, if a solution of NaCl in water is said to be 10 % by volume that means a 100 ml solution will contain 10 ml NaCl.

4. Mass by Volume Percentage (w/V):

This unit is majorly used in the pharmaceutical industry. It is defined as the mass of a solute dissolved per 100mL of the solution.

5. Molarity (M):

One of the most commonly used methods for expressing the concentrations is molarity. It is the number of moles of solute dissolved in one litre of a solution. Suppose a solution of ethanol is marked 0.25 M, this means that in one litre of the given solution 0.25 moles of ethanol is dissolved.

6. Molality (m):

Molality represents the concentration regarding moles of solute and the mass of solvent. It is given by moles of solute dissolved per kg of the solvent**.** The molality formula is as given-

Molality(m)=Moles of solute/Mass of solvent in kg Or

$$Molality(m) = \frac{Moles\ of\ solute}{Mass\ of\ solvent\ in\ kg}$$

7. Normality

It is the number of gram equivalents of solute present in one liter of the solution and it is denoted by N.

$$N = \frac{Weight\ of\ solute\ in\ grams}{Equivalent\ mass \times Volume\ in\ liter}$$

N=Weight of solute in grams/Equivalent mass × Volume in liter

The relation between normality and molarity.

- N x Eq.Wt = Molarity x Molar mass
- N = Molarity x Valency
- N = Molarity x Number of H^+ or OH^- ion.

8. Formality

It is the number of gram formula present in one litre of solution. It is denoted by F.

$$F = \frac{Weight\ of\ solute\ in\ gram}{Formula\ wt \times Volume\ in\ liter}$$

F=Weight of solute in gram/Formula wt.×Volume in liter
It is applicable in the case of ionic solids like NaCl.

9. Mole Fraction:

If the solution has a solvent and the solute, a mole fraction gives a concentration as the ratio of moles of one component to the total moles present in the solution. It is denoted by x. Suppose we have a solution containing As a solute and B as the solvent. Let n_A and n_B be the number of moles of A and B present in the solution respectively. So mole fractions of A and B are given as:

$$x_A = \frac{n_A}{n_A + n_B}$$
$$x_B = \frac{n_B}{n_A + n_B}$$

The above-mentioned methods are commonly used ways of expressing the concentration of solutions. All the methods describe the same thing that is, the concentration of a solution, each of them has its own advantages and disadvantages. Molarity depends on temperature while mole fraction and molality are independent of temperature. All these methods are used on the basis of the requirement of expressing the concentrations.

Solutions of Solids in Liquids

1. A saturated solution is a solution that remains in contact with an excess of solute.
2. The amount of solute dissolved per 100g of solvent in a saturated solution at a specific temperature represents the solubility of the solute.
3. For exothermic substances such as KOH, CaO, $Ca(OH)_2$, M_2CO_3, M_2SO_4, etc, solubility is inversely proportional to temperature.
4. For endothermic substances such as NaCl, KNO_3, $NaNO_3$, glucose, etc., solubility is directly proportional to temperature.

Solubility of Gases

The solubility of gases is mostly expressed in terms of the absorption coefficient,k that is the volume of the gas dissolved by unit volume of solvent at 1 atm pressure and a specific temperature.

The solubility of a gas in a liquid depends upon

1. ***Temperature solubility*** is inversely proportional to temperature as the dissolution of gas is exothermic in most cases.
2. ***Nature of gas –*** Gases having a higher value of van der Waals force of attraction that is gases that are more easily liquefied are more soluble. For example, SO_2 and CO_2 are more soluble in water than O_2, N_2, and H_2.
3. ***Nature of solvent –*** Gases which can ionize in aqueous solution are more stable in water as compared to the other solvents.

UNIT I

Chapter ... 1

PHARMACEUTICAL ANALYSIS

♦ LEARNING OBJECTIVES ♦

- *The course intends to impart knowledge of the basic analytical techniques involved in determining unknown concentrations of different constituents present in solutions or solids using volumetric titrations or gravimetric methods.*
- *To study the preliminaries and definitions used in analysis.*
- *To study the accuracy and precision parameters and minimization of errors during analytical experiments.*

OUTCOMES

On satisfying the requirements of this course, students will have the knowledge and skills to:

- Understand that all measurements involve errors and that the analytical procedure is only as accurate as the least accurate measurement.
- Understand that for each technique the sources and magnitudes of errors can be identified and quantified (If possible); and finally errors can be reduced upto great extent.
- Understand that the magnitude of the error in the analysis can be reflected using the correct significant figures when reporting results.
- Have complete knowledge of basic terms like molarity, molality, normality, weight percentage calculation and applications based on them.

1.1 DEFINITION

Pharmaceutical analysis or chemical analysis is 'a technique to identify and/or quantify any sample, substance, compound by using manual, chemical or instrumental techniques by qualitative or quantitative method(s)'. Analytical chemistry is apprehensive with the theory and practice of techniques used to determine the composition of substance(s). Analytical chemistry is very broad and squeezes a wide range of techniques. Chemical analysis is applied in daily life i.e. soaps and detergents act as emulsifiers to surround dirt and grime so it can be washed away from clothing, dishes and our bodies. Analytical chemistry can be classified into qualitative and quantitative analysis.

1.1.1 Types of Analytical Chemistry

1. **Qualitative Analysis:** Completely unknown sample is analysed to determine the presence or absence of the particular substance(s) in the sample, and the technique is known as qualitative analysis. Qualitative analysis is based on either present or absent phenomenon. Identification of the compounds or substances takes place in this technique.

   Example: Phenolphthalein indicator gives light pink colour in alkaline medium; light pink colour in the alkaline solution will be either present or absent. The technique is qualitative analysis because we are not determining the intensity of colour present.

2. **Quantitative Analysis:** Determination of the quantity in numbers, weight, length or any other measurement parameter is carried out under quantitative analysis. Specified components are quantified in the sample by using quantitative methods. Various quantitative methods are available to determine the samples(s). i.e.: Titrimetry, volumetry gravimetry, thermal, electro-chemical, spectral analysis etc.

   Example: Assay of NaCl is carried out by volumetric method and the accurate percentage purity of the given sample is determined by volumetric analysis.

1.1.2 Steps in Chemical Analysis

1. Sampling

An analyst has to determine the nature of a large quantity of substances(s). In ideal condition analyst should analyze every part of the substance(s) to get the accurate result, but it is not possible every time. So, according to the physical nature and size of the substance(s) available sampling is carried out.

2. Sample preparation

Sample preparation includes the dealing of the samples prior to its analysis i.e. reduction of the particle size, mixing of the samples to get homogeneity, drying of the samples determination of quantity of the sample in terms of weight or volume etc.

3. Processing of sample for analysis

Accurate and above selected sample(s) are processed to carry out the analysis i.e. heating, fusion or ignition of the sample(s), use of the solvent(s) to dissolve the sample(s) dilution of the sample(s) etc. In second step of the sample processing the prepared sample(s) are treated by suitable method to purify, to get better and accurate results i.e. filtration solvent extraction, ion exchange methods, chromatographic separations etc.

4. Selection of analytical technique(s)

Selection of appropriate method was done by the analyst, and basis of the selection is nature of the sample and required output. Various analytical methods are available for analysis of the samples. i.e. volumetric, gravimetric, spectroscopic, electro-chemical etc.

5. Analysis of sample by selected technique

Appropriate analytical method is selected and samples are analysed by selected method to get the result(s). Analysis of the sample involves calibration, optimization, standardization

and measurement of the substances(s) by the selected method. Sometimes validation of the instrumental method is also carried out as per the available guidelines.

6. **Calculation and results**

Analytical data available after the analysis is further processed for calculation, results are compiled and conclusion of the analytical method is given.

7. **Presentation of data**

Processed data and results are represented in the form of tables, graphs and other suitable representation methods.

1.1.3 Factors Affecting the Choice of Analytical Technique

Section of appropriate analytical technique is done by considering many important factors which are:

- **Nature of the sample:** Acid-base sample requires acid-base titration, oxidizing-reducing sample requires redox titration etc.
- **Type of the analysis:** Volumetric analysis, spectroscopic analysis, chromatographic analysis.
- **Physical state of the sample:** Physical state of the sample plays a major role for selection of the analytical technique i.e. solid, liquid and gaseous samples.
- **Presence of the impurities:** Presence of impurities affects the selection of the analytical technique because some analytical methods are not sensitive enough to determine the magnitude of impurities present in the sample.
- **Concentration of the analyte in the sample:** Selection of the analytical technique is affected by the concentration range of the analyte; some analytical techniques are less sensitive and some are more sensitive. So, more sensitive analytical techniques are selected for the samples containing lower concentration range of the analyte.
  Example: pH of the solution can be determined by pH paper and pH meter; but pH meter is highly sensitive and can be differentiating between the two solutions of very low concentration range difference.
- **Accuracy required:** Accuracy of the different analytical techniques varies. For more accurate results highly sensitive techniques are used.
- **Availability of the instrument:** Availability of the instrument(s) for selected analytical technique plays major role. If instrument(s) are not available for the selected technique then the method is useless.
- **Time required for the analysis:** Time required to complete the analysis is the major factor for selection of the technique. If result is required fast then appropriate method is selected to get the results immediately.
- **Amount of the sample available:** If amount of the sample is not available in sufficient quantity then non-destructive method is preferred (if available).

- **Cost of the analysis:** Selection of the analytical technique is highly governed by the cost of the analysis. Low cost analytical techniques are always preferable, if high cost analytical techniques are not essential.

1.1.4 Classification of Pharmaceutical Analysis Laboratories

Pharmaceutical analysis laboratories are classified into five major categories; which are:

1. **Government regulatory agencies**

Various government regulatory agencies are established by the central and state government; these agencies are continuously monitoring and analysing the various drug samples.

Example: IPC (Indian Pharmacopoeial Commission), CDSCO (Central Drugs Standard Control Organization) etc.

2. **Manufacturers of drugs**

Large number of drug manufacturing units of government and non-government sectors are equipped with various analytical instruments to analyze the drugs.

3. **Manufacturer of raw materials of drugs**

Large number of raw materials of drugs are manufactured by various industries and these raw materials are analysed in the analytical laboratories present in that industry.

4. **Universities and non-commercial research centres**

Universities, colleges and other non-commercial research centres have their instrumentation laboratory to carry out the analysis of different type of samples.

5. **Consulting laboratories**

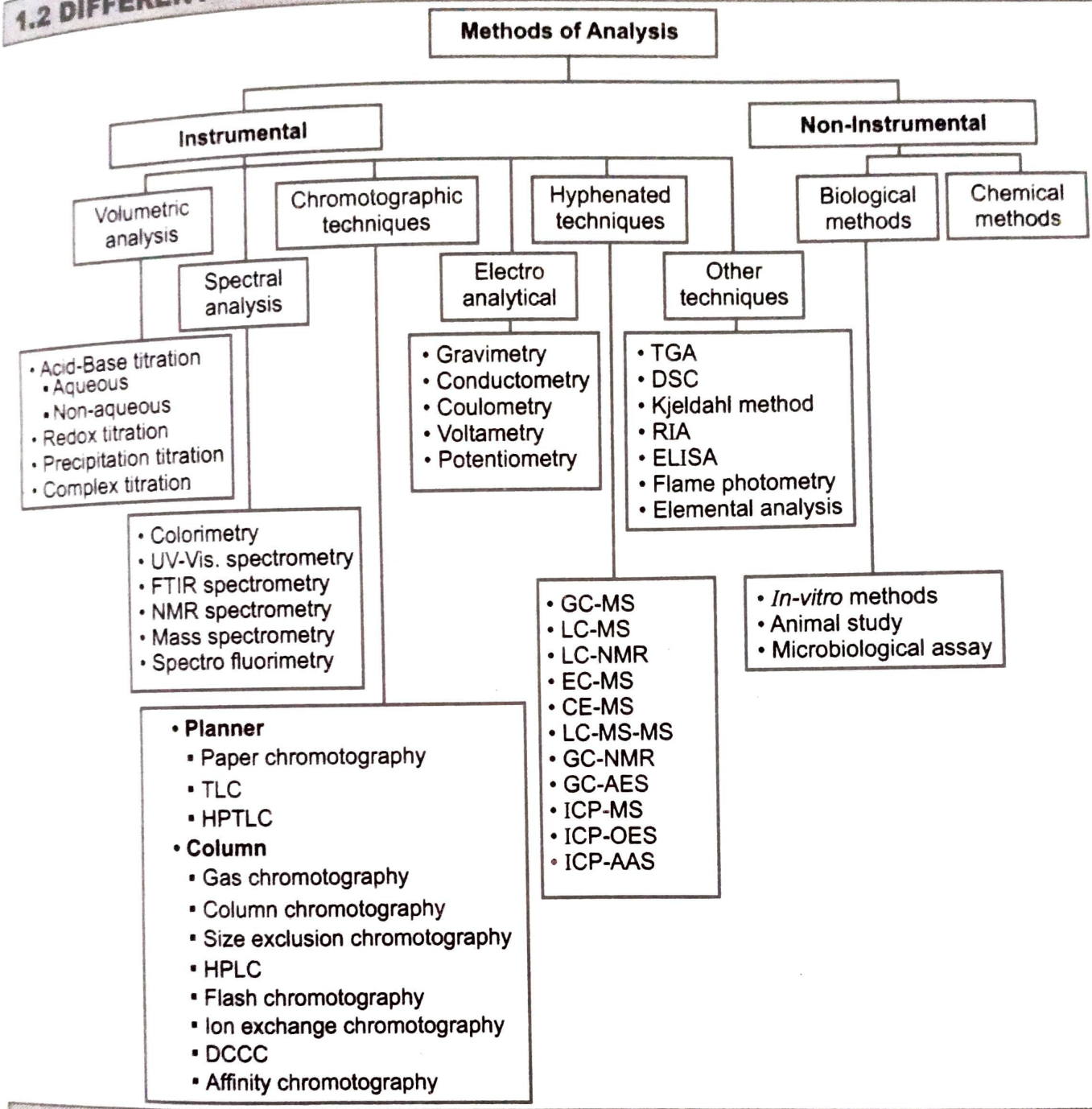
Varieties of consulting laboratories are available to carry out the analysis of the samples by charging on the basis of per sample.

1.1.5 Scope of Analytical Chemistry

The final manufactured product is subjected to quality control to ensure the claimed quantity of the substance(s)

- Examination of raw materials.
- Development of new products.
- Examination of various drug products.
- Qualitative and quantitative analysis of various samples.
- Diagnosis of disease by various chemical analyses.
- Determination or examination of soil and rock.
- Analysis of different samples of water.
- Determination of radioactive compounds.
- Determination of naturally occurring phytoconstituents.

1.2 DIFFERENT TECHNIQUES OF ANALYSIS



1.3 METHODS OF EXPRESSING CONCENTRATION

- In all the techniques of qualitative analysis, the use of solutions require some bases for the expression of solution concentration.
- All the systems of concentration expression have fundamentally similar bases with respect to weight relationships of solute and solvent, but actual method of expression of concentration should take on some convenient and specific form.

(1) Normality:

The normality of a solution is defined as 'the number of gram equivalent present per litre of the solution'. It is denoted by "N".

$$\text{Normality, N} = \frac{\text{Number of gram equivalents}}{\text{Volume (in litre)}}$$

Gram Equivalent (Equivalent weight):

- Equivalent weight of an acid is that weight of it, which contains 1 gm of replaceable hydrogen ion.

$$\text{Equivalent weight for acid} = \frac{\text{Molecular weight}}{\text{Basicity}}$$

- Equivalent weight of monobasic acid is equal to its molecular weight.
- Equivalent weight of a dibasic or a tribasic acid is 1/2 and 1/3 respectively of its molecular weight.
- Similarly, Equivalent weight of a base is the weight of the substance which contains one replaceable hydroxyl group.

OR $$\text{Equivalent weight for base} = \frac{\text{Molecular weight}}{\text{Acidity}}$$

Example:

$$HCl_{\text{equivalent weight}} = \frac{\text{Moleculer weight}}{\text{Basicity}}$$

$$= \frac{36.5}{1}$$

$$= 36.5$$

$$H_2SO_{4\text{equivalent weight}} = \frac{\text{Molecular Weight}}{\text{Basicity}}$$

$$= \frac{98}{2}$$

$$= 49$$

$$NaOH_{\text{equivalent weight}} = \frac{\text{Molecular Weight}}{\text{Acidity}}$$

$$= \frac{40}{1}$$

$$= 40$$

$$Na_2CO_{3\text{equivalent weight}} = \frac{\text{Molecular Weight}}{\text{Acidity}}$$

$$= \frac{106}{2}$$

$$= 53$$

(2) Percent Concentration:

Concentration is many-a-times expressed in terms of percent (parts per hundred). Percent composition of a solution can be expressed as:

$$\%\,\frac{w}{w} = \frac{\text{Weight of solute (in gm)}}{\text{Weight of solution (in gm)}} \times 100$$

Example: Preparation of 10% w/w NaCl solution (10 g of NaCl will be dissolved in 90 g of distilled water).

$$\%\ \frac{v}{v} = \frac{\text{Volume of solute}}{\text{Volume of solution}} \times 100$$

Example: Preparation of 10% v/v acetic acid (10 ml of glacial acetic acid will be dissolved in distilled water and final volume of the solution will be 100 ml).

$$\%\ \frac{w}{v} = \frac{\text{Weight of solute (in gm)}}{\text{Volume of solution}} \times 100$$

Example: Preparation of 10% w/v NaCl solution (10 g of NaCl will be dissolved in distilled water and final volume of the solution will be 100 ml).

(3) Molal Concentration:

It is rarely used in analytical technique.

Molality of the solution is given by 'the number of moles of solute per kg of solvent'. It is represented by 'm' and is independent of the temperature.

$$m = \frac{\text{Number of moles of solute}}{\text{Volume of solvent (in kg)}}$$

Example: Preparation of 1 m NaOH solution (40 g of NaOH is dissolved in 1 kg of distilled water).

(4) Molar Concentration:

The molar concentration of the solution is 'the number of moles of solute per litre of solution'. It is represented by 'M'.

$$M = \frac{\text{Number of moles of solute}}{\text{Volume of solution (in litre)}}$$

Volume may be changed due to change in temperature, so it is temperature dependent.

Example: Preparation of 1 M NaOH solution (40 g of NaOH is dissolved in distilled water and the final volume is made up to 1000 ml).

(5) Formal Concentration:

Some substances do not exist in molecular form whether in solid or solution form. They remain in ionic form in solid state as well as in solution. In such cases, formula weight is used in preparation of solution and its concentration is expressed in terms of formality. The difference between formal and molar is that, the formal concentration indicates moles of the original chemical formula in solution, without regard for the species that actually exist in solution. Molar concentration, on the other hand, is the concentration of species in solution.

Example: NaCl, $FeCl_3$ can be defined very simply as 'number of formula weight of a solute per litre of solution'.

$$F = \frac{\text{Number of moles of solute}}{\text{Volume of solution (in litre)}}$$

Formal solutions generally show changes in formality where volume changes are associated with temperature.

Example: Preparation of 1 F NaCl solution (Dissolve 58.5 g of NaCl in distilled water and make up the final volume upto 1000 ml).

(6) Parts per million (ppm):

Parts per million is frequently used to express the concentration of very dilute solutions and is expressed as 'ppm'. These terms are also employed to express the concentration of impurities in pharmaceuticals. Parts per billion (ppb) is very rarely used.

$$C_{ppm} = \frac{\text{Mass of solute (in gm)}}{\text{Mass of solution (in gm)}} \times 10^6 \text{ ppm}$$

Example: Prepare 100_{ppm} solution of chloride from NaCl.

$$100_{ppm} = \frac{100 \times 100\%}{1000000}$$

$$= 0.01\%$$

Molecular weight of NaCl = 58.5 and Molecular weight of Cl^- = 35.5

So, $\frac{58.5 \times 0.01}{35.5}$ = 0.01648 g of NaCl is dissolved in 100 ml of distilled water

1.4 PRIMARY AND SECONDARY STANDARD SUBSTANCES

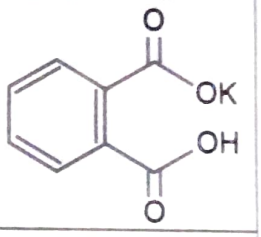
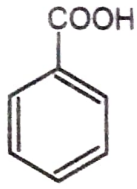
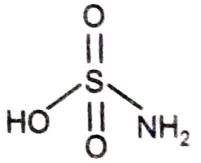
- Very pure reagents are used for standardization.
- A solution with accurately known concentration is called standard solution. Accurate weight of the selected chemical or reagent is taken, dissolved and diluted to make the required volume.
- Highly pure reagents or chemicals are used to prepare standard solution which does not require further standardization, is known as primary standard solution.

1.4.1 Primary Standard Substances

- Primary standard substance should be easily available, purified and dried.
- These substances should be 100% pure; if impurities are present then magnitude of impurities should be accurately known to the analyst. Magnitude of impurities should not exceeds 0.01 - 0.02%
- Primary standard substance should be stable at normal atmospheric conditions and free from any hygroscopic properties.
- Equivalent weight and molecular weight of these substances should be high to reduce the weighing errors as higher weight leads to reduction in the weighing error.
- Reaction of the standard solution with the reactant should be instant and stoichiometric for easy calculations.
- Theoretical equivalence point and practical end point should be equal.
- Primary standard substance should be completely soluble under the experimental conditions.

- Primary standard substance should be free from any hydrated water moiety.
- Primary standard substances will not be able to satisfy all the above mentioned conditions, so closely relevant substances are considered as primary standard.

Table 1.1: List of Commonly used Primary Standards

Sr. No.	Name	Molecular Weight	Formula / Structure
I.	**Acid Base Titration:**		
	1. Sodium carbonate	105.99	Na_2CO_3
	2. Sodium tetraborate (Borax)	381.37	$Na_2B_4O_7 \cdot 10H_2O$
	3. Oxalic acid	126.07	$C_2H_2O.\ 2H_2O$
	4. Potassium hydrogen phthalate	204.22	
	5. Succinic acid	118.08	CH_2COOH | CH_2COOH
	6. Benzoic acid	122.12	
	7. Adipic acid	146.14	CH_2CH_2COOH | CH_2CH_2COOH
	8. Sulphamic acid	97.10	
	9. Constant boiling HCl can also be used as a primary standard	36.46	HCl
II.	**Redox Titration:**		
	1. Potassium dichromate	294.18	$K_2Cr_2O_7$
	2. Potassium bromate	167.00	$KBrO_3$
	3. Potassium iodate	214.00	KIO_3
	4. Potassium hydrogen iodate	389.91	$KH(IO_3)_2$
	5. Sodium oxalate	134.00	$Na_2C_2O_4$
	6. Arsenic trioxide	197.84	As_2O_3
	7. Copper sulphate	249.68	$CuSO_4.5H_2O$

Sr. No.	Name	Molecular Weight	Formula / Structure
III.	**Precipitation Titrations:**		
	1. Silver	107.87	Ag
	2. Silver nitrate	169.87	$AgNO_3$
	3. Sodium chloride	58.44	NaCl
	4. Potassium chloride	74.55	KCl
	5. Potassium Bromide	119.00	KBr
IV.	**Complexometric Titration:**		
	1. Metallic Zinc	65.38	Zn
	2. Metallic Magnesium	24.31	Mg
	3. Zinc chloride	136.29	$ZnCl_2$
	4. Calcium chloride	110.98	$CaCl_2$

1.4.2 Secondary Standard Substances

- There are the substances used for the standardization and whose concentration has been determined by comparison with the primary standards.

  Example: An unknown solution of HCl can be determined by two methods.

  (a) By using analytical grade Na_2CO_3 (Primary Standards).

  (b) By using standard solution of NaOH (Secondary Standard).

1.5 PREPARATION AND STANDARDIZATION OF VARIOUS MOLAR AND NORMAL SOLUTIONS

1. Oxalic Acid:

Aim: To prepare 0.1 N oxalic acid standard solution.

Requirements: Oxalic acid, volumetric flask, distilled water.

Oxalic acid is primary standard substance; so it does not requires standardization.

Preparation of 0.1 N Oxalic Acid:

Molecular formula of oxalic acid is $C_2H_2O_4.2H_2O$.

Molecular weight of oxalic acid is 126.

$N = \frac{126}{2}$, N = 63 g/Litre of oxalic acid (for 1 N)

So, 6.3 g/Litre of oxalic acid (for 0.1 N).

Take a 1000 ml volumetric flask and transfer the accurate amount of 6.3 g of oxalic acid and dissolve and 100 ml of distilled and make the final volume upto 1000 ml by using distilled water. Normality of the prepared oxalic acid solution will be 0.1 N.

2. Sodium Hydroxide:

Aim: To prepare a standardized 0.1 N NaOH solution by using 0.1 N HCl.

Requirements: Concentrated hydrochloric acid, sodium carbonate, sodium hydroxide, distilled water, methyl orange indicator, burette, pipette, volumetric flask, beaker and funnel

Principle:

Step 1: Standardization of HCl:

Titration between hydrochloric acid and sodium carbonate is based on the principle of strong acid and strong base titration.

$$Na_2CO_3 + 2HCl \longrightarrow 2NaCl + CO_2 + H_2O$$

This reaction proceeds into two stages:

$$Na_2CO_3 + HCl \longrightarrow NaHCO_3 + NaCl$$

$$NaHCO_3 + HCl \longrightarrow H_2O + CO_2 + NaCl$$

Step 2: Standardization of NaOH:

Titration between sodium hydroxide and standardized hydrochloric acid is based on the principle of strong acid and strong base titration.

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

Preparation of Reagents:

Preparation of 0.1 N HCl:

Concentrated HCl has approximately 11.5 *N* Strength.

$$N_1V_1 = N_2V_2$$

Here, $N_1 = 11.5$, $V_1 = ?$, $N_2 = 0.1$, $V_2 = 1000$ ml

So, $11.5 \times V_1 = 0.1 \times 1000$

$V_1 = 8.70$ ml

Preparation of 0.1 N Sodium carbonate:

$N = \frac{106}{2}$, N = 53 g/Litre of sodium carbonate (for 1 N)

So, 5.3 g/Litre of sodium carbonate (for 0.1 N)

Preparation of 0.1 N Sodium hydroxide:

$N = \frac{40}{1}$, N = 40 g/Litre of sodium hydroxide (for 1 N)

So, 4.0 g/Litre of sodium hydroxide (for 0.1 N)

Experimental Methodology:

1. Take approximate 200-300 ml of distilled water into a 1000 ml volumetric flask and slowly add 8.7 ml concentrated hydrochloric acid dropwise in the volumetric flask and finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be approximate 0.1 N; which needs standardization because hydrochloric acid is a secondary standard substance.
2. Take 5.3 g of sodium carbonate in a 1000 ml volumetric flask and add approximate 100 ml of distilled water and dissolve; finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be 0.1 N because sodium carbonate is a primary standard substance.

3. Take 4.0 g of sodium hydroxide in a 1000 ml volumetric flask and add approximate 100 ml of distilled water and dissolve; finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be approximately 0.1 N; which needs standardization because sodium hydroxide is a secondary standard substance.
4. *Standardization of 0.1 N HCl:*
   Transfers 10 ml of the prepared hydrochloric acid solution, with a pipette, to a conical flask. Then add one or two drops of methyl orange indicator to this solution.
   Take the sodium carbonate solution in a burette and add gradually with continuous swirling of the solution in the conical flask; near the end point, the sodium carbonate solution is added drop by drop. Continue the addition of the sodium carbonate until the colour of the solution turns to orange from red and finally orange to yellow at the end point.
   Repeat the experiment three or more times until two consecutive results are same or precise and tabulate the results. Take the precise readings for calculation of actual normality.
5. *Standardization of 0.1 N NaOH:*
   Transfer 10 ml of the prepared sodium hydroxide solution, with a pipette, to a conical flask then add one or two drops of phenolphthalein indicator to this solution.
   Take the standardized HCl solution in a burette and add gradually with continuous swirling of the solution in the conical flask; near the end point, HCl is added drop by drop. Continue the addition of the HCl until the colour of the solution turns to colourless from pink.
   Repeat the experiment three or more times until two consecutive results are same or precise and tabulate the results. Take the precise readings for calculation of actual normality

Observation Table:

Standardization of HCl:

Sr. No.	Start Point	End Point	Volume Consumed
1.			
2.			
3.			
4.			
5.			

Standardization of NaOH:

Sr. No.	Start Point	End Point	Volume Consumed
1.			
2.			
3.			
4.			
5.			

Calculation:

Standardization of 0.1 N HCl:

$$N_1V_1 = N_2V_2$$

Here,

N_1 (Normality of sodium carbonate) = 0.1 N

V_1 (Volume of sodium carbonate consumed) = x_1 ml

N_2 (Normality of hydrochloric acid) = y N

V_2 (Volume of hydrochloric acid taken) = 10 ml

Standardization of 0.1 N NaOH:

$$N_2V_2 = N_3V_3$$

Here,

N_2 (Calculated normality of hydrochloric acid) = y N

V_2 (Volume of hydrochloric acid consumed) = x_2 ml

N_3 (Normality of sodium hydroxide) = z N

V_3 (Volume of sodium hydroxide taken) = 10 ml

Result:

Sodium hydroxide solution was prepared and standardized using the standard y N hydrochloric acid; and normality of the prepared sodium hydroxide solution was found to be z N.

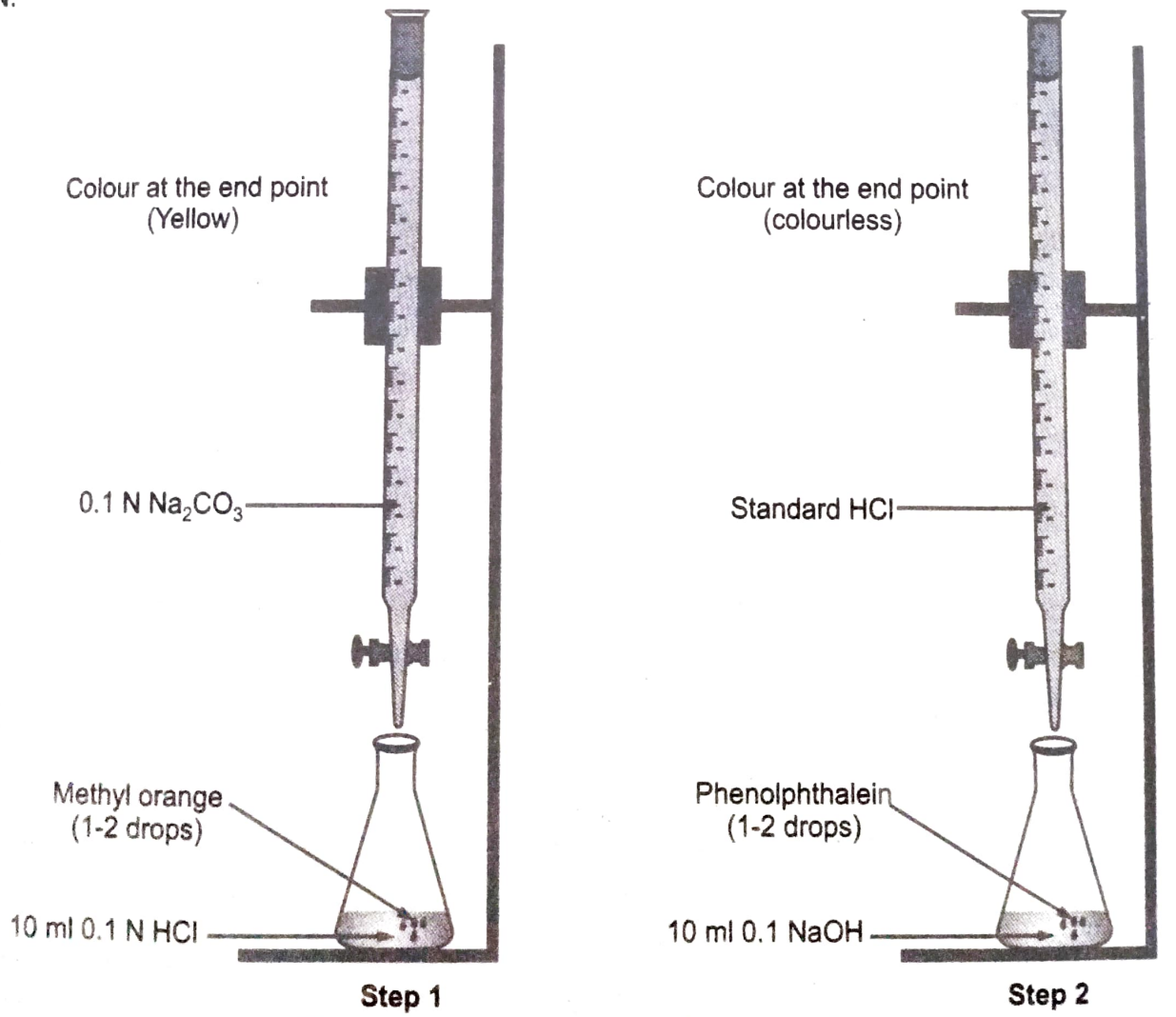


Fig. 1.1: Standardization of 0.1 N NaOH

3. Hydrochloric Acid:

Aim: To prepare a standardized 0.1 N HCl solution by using primary standard 0.1 N HCl.

Requirements: Concentrated hydrochloric acid, sodium carbonate, sodium hydroxide, distilled water, methyl orange indicator, burette, pipette, volumetric flask, beaker, funnel.

Principle: Titration between hydrochloric acid and sodium carbonate is based on the principle of strong acid and strong base titration.

$$Na_2CO_3 + 2HCl \longrightarrow 2NaCl + CO_2 + H_2O$$

This reaction proceeds into two stages:

$$Na_2CO_3 + HCl \longrightarrow NaHCO_3 + NaCl$$
$$NaHCO_3 + HCl \longrightarrow H_2O + CO_2 + NaCl$$

Preparation of Reagents:

Preparation of 0.1 N HCl:

Concentrated HCl has approximately 11.5 N Strength.

$$\therefore \quad N_1V_1 = N_2V_2$$

Here, $N_1 = 11.5$, $V_1 = ?$, $N_2 = 0.1$, $V_2 = 1000$ ml

So, $$11.5 \times V_1 = 0.1 \times 1000$$
$$V_1 = 8.70 \text{ ml}$$

Preparation of 0.1 N Sodium carbonate:

$N = \frac{106}{2}$, N = 53 g/Litre of sodium carbonate (for 1 N)

So, 5.3 g/Litre of sodium carbonate (for 0.1 N).

Experimental Methodology:

1. Take approximate 200-300 ml of distilled water into a 1000 ml volumetric flask and slowly add 8.7 ml concentrated hydrochloric acid dropwise in the volumetric flask and finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be approximate 0.1 N; which needs standardization because hydrochloric acid is a secondary standard substance.
2. Take 5.3 g of sodium carbonate in a 1000 ml volumetric flask and add approximate 100 ml of distilled water and dissolve; finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be 0.1 N because sodium carbonate is a primary standard substance.
3. Transfer 10 ml of the prepared hydrochloric acid solution, with a pipette, to a conical flask then add one or two drops of methyl orange indicator to this solution.
4. Take the sodium carbonate solution in a burette and add gradually with continuous swirling of the solution in the conical flask, and near the end point, the sodium carbonate is added drop by drop. Continue the addition of the sodium carbonate until the colour of the solution turns to orange from red and finally orange to yellow at the end point.
5. Repeat the experiment three or more times until two consecutive results are same or precise and tabulate the results.
6. Take the precise readings for calculation of normality.

Observation Table:

Sr. No.	Start Point	End Point	Volume Consumed
1.			
2.			
3.			
4.			
5.			

Calculation:

$$N_1V_1 = N_2V_2$$

Here,

N_1 (Normality of sodium carbonate) = 0.1 N

V_1 (Volume of sodium carbonate consumed) = x ml

N_2 (Normality of hydrochloric acid = y N

V_2 (Volume of hydrochloric acid taken) = 10 ml

Result:

Hydrochloric acid solution was prepared and standardized using the standard 0.1 N sodium bicarbonate solution; actual normality of the solution was found to be y N.

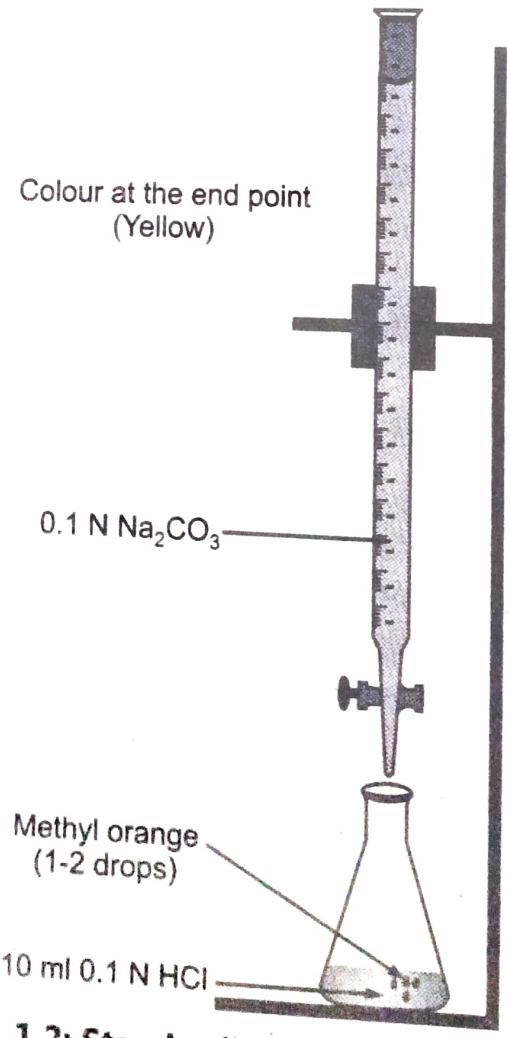


Fig. 1.2: Standardisation of 0.1 N HCl

4. Sodium Thiosulphate:

Aim: To prepare a standardized 0.1 M $Na_2S_2O_3$ solution by using primary standard potassium dichromate.

Requirements: Sodium thiosulphate pentahydrate, potassium dichromate, distilled water, burette, pipette, volumetric flask, beaker, funnel.

Principle: Standardization of sodium thiosulphate is based on the iodometric method. Iodine oxidizes thiosulphate to the tetrathionate ion:

$$I_2 + 2S_2O_3^{2-} \longrightarrow 2I^- + S_4O_6^{2-}$$

Preparation of Reagents:

Preparation of 0.1 M $Na_2S_2O_3$:

Dissolve about 24.8 g of $Na_2S_2O_3.5H_2O$ crystals in 1000 ml of recently boiled and cooled water; add about 0.2 g of sodium carbonate as a preservative and store in a clean bottle.

Experimental Methodology:

1. Accurately weigh 0.21 g of potassium dichromate (previously dried at 120°C for 4 hours) and dissolve in 100 ml distilled water; add 3 g of potassium iodide, 2 g sodium bicarbonate and 5 ml concentrated HCl. Swirl the solution and keep in dark for 10 minutes in iodine flask.
2. Take the above prepared solution in iodine flask and titrate the liberated iodine with prepared sodium thiosulphate solution until the colour changes to yellowish green.
3. Add 3 ml of starch indicator solution and carry out the titration until the blue colour disappears.
4. Calculate the molarity of the solution.

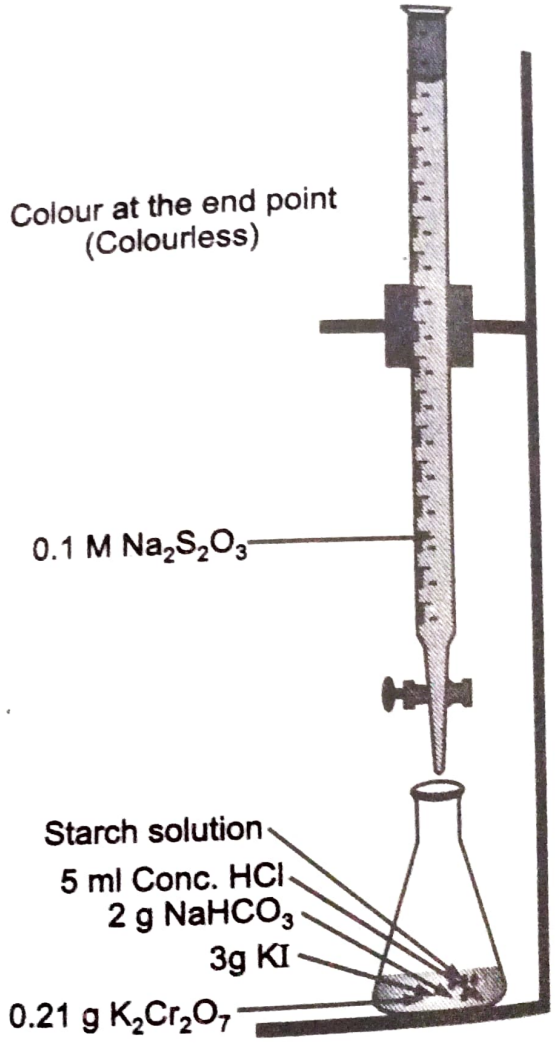


Fig. 1.3: Standardization of 0.1 M $Na_2S_2O_3$

Calculation:

$$\text{Molarity of sodium thiosulphate} = \frac{\text{Potassium dichromate (in mg)}}{49.04 \times \text{Sodium thiosulphate (in ml)}}$$

Result:

Sodium thiosulphate solution was prepared and standardized and actual molarity of the solution was found to be y M.

5. Sulphuric Acid:

Aim: To prepare a standardized 0.1 N H_2SO_4 solution by using primary standard 0.1 N Na_2CO_3.

Requirements: Concentrated sulphuric acid, sodium carbonate, distilled water, methyl orange indicator, burette, pipette, volumetric flask, beaker, funnel.

Principle: Titration between hydrochloric acid and sodium carbonate is based on the principle of strong acid and strong base titration.

$$Na_2CO_3 + H_2SO_4 \longrightarrow Na_2SO_4 + CO_2 + H_2O$$

Preparation of Reagents:

Preparation of 0.1 N H_2SO_4:

Concentrated H_2SO_4 has approximately 36.5 N Strength.

$$N_1V_1 = N_2V_2$$

Here, $N_1 = 36.5$, $V_1 = ?$, $N_2 = 0.1$, $V_2 = 1000$ ml

So, $36.5 \times V_1 = 0.1 \times 1000$

$V_1 = 2.74$ ml

Preparation of 0.1 N sodium carbonate:

$N = \frac{106}{2}$, N = 53 g/Litre of sodium carbonate (for 1 N)

So, 5.3 g/Litre of sodium carbonate (for 0.1 N)

Experimental Methodology:

1. Take approximate 200-300 ml of distilled water into a 1000 ml volumetric flask and slowly add 2.74 ml concentrated sulphuric acid dropwise in the volumetric flask and finally make up the volume up to 1000 ml by distilled water. Normality of the prepared solution will be approximate 0.1 N which needs standardization because sulphuric acid is a secondary standard substance.
2. Take 5.3 g of sodium carbonate in a 1000 ml volumetric flask and add approximate 100 ml of distilled water and dissolve; finally make up the volume upto 1000 ml by distilled water. Normality of the prepared solution will be 0.1 N because sodium carbonate is a primary standard substance.
3. Transfers 10 ml of the prepared sulphuric acid solution, with a pipette, to a conical flask then add one or two drops of methyl orange indicator to this solution.
4. Take the sodium carbonate solution in a burette and add gradually with continuous swirling of the solution in the conical flask, and near the end point, the sodium carbonate is added drop by drop. Continue the addition of the sodium carbonate until the colour of the solution turns to orange from red and finally orange to yellow at the end point.

5. Repeat the experiment three or more times until two consecutive results are concordant, precise and tabulate the results.
6. Take the precise readings for calculation of normality.

Observation Table:

Sr. No.	Start Point	End Point	Volume Consumed
1.			
2.			
3.			
4.			
5.			

Calculation:

$$N_1V_1 = N_2V_2$$

Here,

N_1 (Normality of sodium carbonate) = 0.1 N
V_1 (Volume of sodium carbonate consumed) = x ml
N_2 (Normality of sulphuric acid) = y N
V_2 (Volume of sulphuric acid taken) = 10 ml

Result:

Hydrochloric acid solution was prepared and standardized using the standard 0.1 N sodium bicarbonate solution; actual normality of the solution was found to be y N.

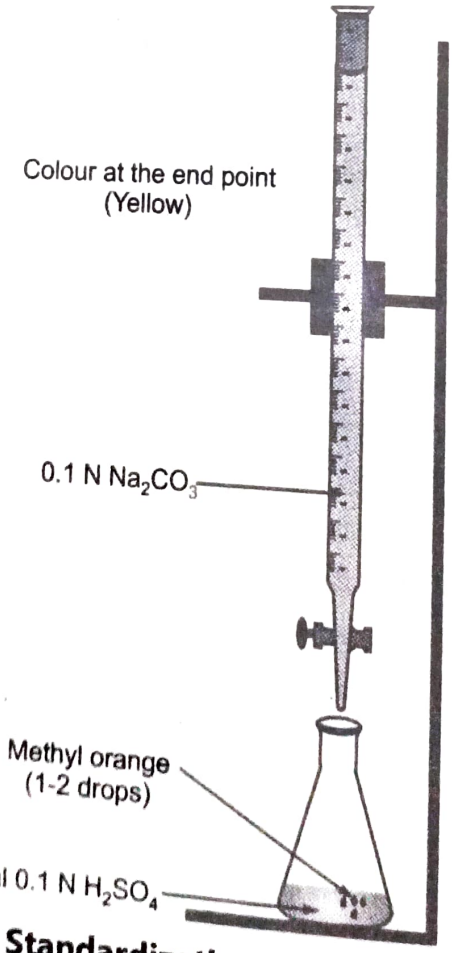


Fig. 1.4: Standardization of 0.1 N H_2SO_4

6. Potassium Permanganate:

Aim: To prepare a standardized 0.1 N $KMnO_4$ solution by using standard solution of 0.1 N oxalic acid.

Requirements: Potassium permanganate, oxalic acid, conc. sulphuric acid, distilled water, burette, pipette, volumetric flask, beaker, funnel.

Principle:

The permanganate ion gets reduced to different products depending upon the reaction conditions. In basic medium, manganate ions are formed, in neutral medium the product is manganese dioxide, whereas in acidic solution, manganous ions are produced as shown by the following equation:

$$MnO_4^- + e^- \rightarrow MnO_4^{2-} \quad \text{(Basic medium)}$$

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \quad \text{(Neutral medium)}$$

$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O \quad \text{(Acidic medium)}$$

The above written equations represent reduction of permanganate ion, but $KMnO_4$ will have different values of equivalent weight in three cases.

$$\text{Equivalent weight of } KMnO_4 \text{ (in basic medium)} = \frac{\text{Molecular weight}}{1} = \frac{158}{1} = 158$$

$$\text{Equivalent weight of } KMnO_4 \text{ (in Neutral medium)} = \frac{\text{Molecular weight}}{3} = \frac{158}{3} = 52.67$$

$$\text{Equivalent weight of } KMnO_4 \text{ (in acidic medium)} = \frac{\text{Molecular weight}}{5} = \frac{158}{5} = 31.6$$

The standardization of potassium permanganate solution is based upon the following equations:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \longrightarrow 3K_2SO_4 + 2MnO_4 + 8H_2O + 10CO_2$$

Preparation of Reagents:

Preparation of 0.1 N $KMnO_4$:

Dissolve about 3.16 g of $KMnO_4$ crystals in 1000 ml distilled water and boil the prepared solution, filter and cool.

Preparation of 0.1 N $H_2C_2O_4.2H_2O$:

Accurately dissolve 6.3 g of oxalic acid crystals in 1000 ml distilled water.

Experimental Methodology:

1. Pipette out 25 ml prepared 0.1 N oxalic acid solution, add 5 ml of concentrated sulphuric acid along the side of the flask, swirl the contents carefully and warm upto 70°C.
2. Titrate the warmed solution against the potassium permanganate solution from the burette till the pink colour persists for about 30 seconds.
3. Repeat the experiment three or more times until two consecutive results are same or precise and tabulate the results.
4. Take the precise readings for calculation of normality

Precautions:

(i) Sufficient acid must be present, otherwise formation of a brown colour during titration may be observed,

(ii) Similar brown colouration can also be observed by using too high a temperature or by using a dirty flask, and

(iii) To avoid such anomalies always rinse the flask with solution of H_2O_2 and dilute H_2SO_4 before performing the titrations.

Observation Table:

Sr. No.	Start Point	End Point	Volume Consumed
1.			
2.			
3.			
4.			
5.			

Calculation:

$$N_1V_1 = N_2V_2$$

Here,

N_1 (Normality of potassium permanganate) = x N

V_1 (Volume of potassium permanganate consumed) = x ml

N_2 (Normality of oxalic acid) = 0.1 N

V_2 (Volume of oxalic acid taken) = 25 ml

Result:

Potassium permanganate solution was prepared and standardized using the standard 0.1 N oxalic acid solution; normality of the solution was found to be x N.

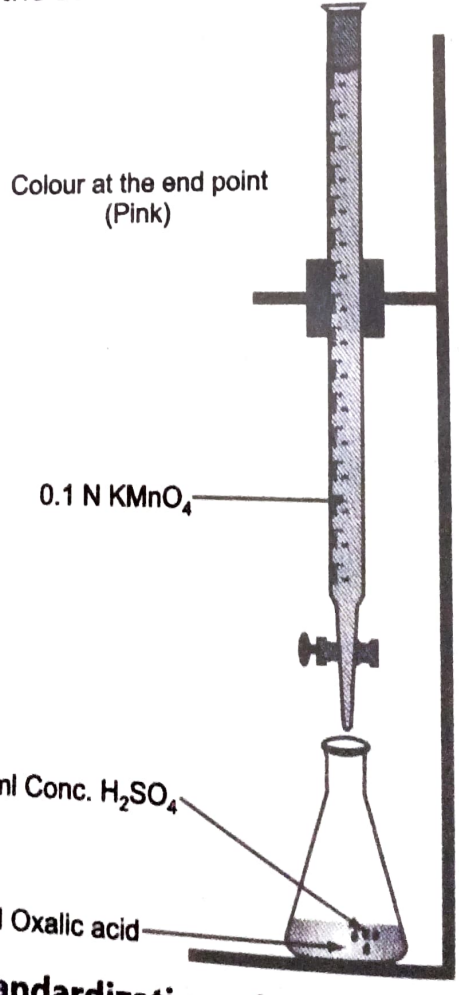


Fig. 1.5: Standardization of 0.1 N $KMnO_4$

7. Cerric Ammonium Sulphate:

Aim: To prepare a standardized 0.1 M cerric ammonium sulphate solution.

Requirements: Cerric ammonium sulphate, concentrated sulphuric acid, arsenic trioxide, sodium hydroxide, osmic acid, ferroin sulphate solution, distilled water, burette, pipette, volumetric flask, beaker, funnel.

Principle:

Cerric ammonium sulphate acts as a potent oxidizing agent in an acidic medium.

$$Ce^{4+} + e^- \longrightarrow Ce^{3+}$$

$$As_2O_3 + 6NaOH \longrightarrow 2Na_3AsO_3 + 3H_2O$$

$$2Ce(SO_4)_2 + Na_3AsO_3 + H_2O \longrightarrow Ce_2(SO_4)_3 + Na_3AsO_4 + H_2SO_4$$

Ferroin acts as indicator in titrations with ceric salts and the reaction is:

$$\underset{\text{Orthophenanthrolin (colourless) base}}{C_{12}H_8N_2} + Fe^{2+} \longrightarrow \underset{\text{Ferrous complex (red)}}{Fe(C_{12}H_8N_2)_3^{2+}} \rightleftharpoons \underset{\text{Ferric complex (blue)}}{Fe(C_{12}H_8N_2)_3^{3+}} + e$$

Preparation of Reagents:

Preparation of 0.1M $H_{20}N_4S_4O_{18}Ce.2H_2O$:

Dissolve about 66 g of $H_{20}N_4S_4O_{18}Ce.2H_2O$ in 500 ml distilled water containing 30 ml concentrated H_2SO_4 with gentle heating. Cool the prepared solution, filter and finally make up the volume upto 1000 ml by distilled water.

Preparation of 8.0% w/v NaOH:

Dissolve accurately weighed 8.0 g of NaOH in 100 ml distilled water.

Preparation of 1.0% w/v OsO_4:

Dissolve accurately weighed 100 mg of OsO_4 in 10 ml distilled water.

Preparation of 10.0% w/v H_2SO_4:

Dissolve accurately weighed 10 g or 5.43 ml of H_2SO_4 in 100 ml distilled water.

Preparation of ferroin sulphate solution:

Dissolve 0.7 g of ferrous sulphate in 70 ml of distilled water and add 1.5 g of 1, 10 phenanthroline and sufficient water to produce 100 ml.

Experimental Methodology:

1. Weigh accurately about 0.2 g of arsenic trioxide (previously dried at 105°C for 1 hour) and transfer to a 500 ml conical flask.
2. Wash the inner walls of the flask with 25 ml of sodium hydroxide solution (8% w/v), swirl to dissolve, add 100 ml of water and mix.
3. Add 30 ml of diluted sulphuric acid (10% w/v), 0.15 ml of osmic acid solution (0.1%), 0.1 ml of ferroin sulphate solution.
4. Titrate the above prepared mixture with cerric ammonium sulphate solution until the pink colour is changed to pale blue.

5. Each 4.946 mg of arsenic trioxide is equivalent to 1 ml of 0.1 N cerric ammonium sulphate or 0.06326 g of $H_{20}N_4S_4O_{18}Ce$.
6. Four equivalents of cerric ammonium sulphate are required to oxidise one mole of arsenic trioxide, hence, 1 equivalent weight of arsenic trioxide is 1/4 mole or 197.84/4 or 49.46 g and 1 milli equivalent shall contain 49.46 mg or 0.04946 g.

Calculation:

$$N = \frac{\text{Weight of arsenic trioxide}}{\text{Volume (in ml)} \times 0.04946}$$

Result:

Cerric ammonium sulphate solution was prepared and standardized using the standard arsenic trioxide solution; normality of the solution was found to be x N.

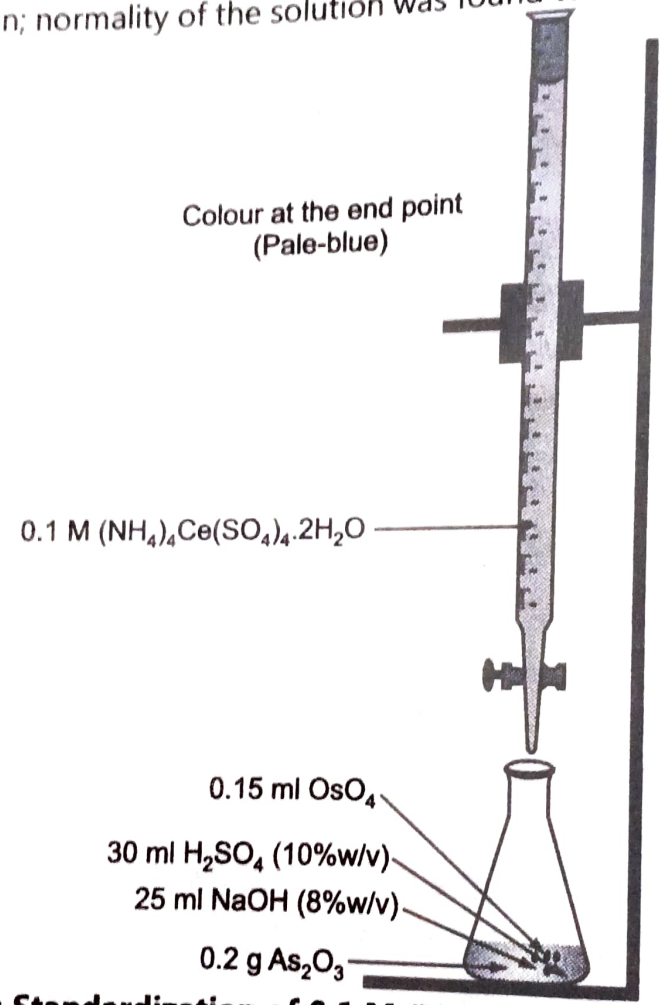


Fig. 1.6: Standardization of 0.1 M $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$

1.6 ERRORS

Error is an action which means mistake. In analytical chemistry, the difference between the true value or standard value and observed value is called error.

Example: If a tablet contains 500 mg of paracetamol and after analysis the analyst observed 490 mg of paracetamol in the tablet.

Then,

$$\text{Absolute Error} = 500 - 490 = 15 \text{ mg}$$

$$\text{Percentage Error} = 100 - [(490/500) \times 100] = 2\%$$

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When any analytical determination is carried out, it is not possible to completely eliminate the error, even when the person making the measurement is an expert working with apparatus of best quality. So, different or repeated attempts are made to minimize the error. Magnitude of the error should be known to the analyst. Since, true values are never known for many determinations, so one has to made use of the most probable or standard value.

1.6.1 Sources of Errors

- **Sample preparation:** Error may occur during preparation of sample.
- **Error by analyst:** Analyst can do error during analysis; it is also known as human error.
- **Equipment problem:** Error may occur due to improper or defective equipment.
- **Calibration:** Error may occur if proper calibration is not done.
- **Reporting error:** Analyst may do errors in writing reports.
- **Calculation error:** Errors may occur during calculation of the results.
- **Error in method selection:** Errors may occur due to wrong method selection.
- **Error during transport and storage:** Error may occur due to improper handling of materials during transport and storage.
- **Sampling error:** Errors may occur due to improper sampling.
- **Laboratory environment:** Errors may occur if suitable laboratory environment is not available for analysis.

1.6.2 Types of Errors

1. Determinate Errors

Determinate errors are also known as systematic errors and cause(s) of these errors are known to the analyst. These are usually one sided and by preplanning and careful working, can be avoided or kept at minimum. These are errors that have a distinct esteem together with a sensible assignable cause. In any case, on a basic level these avoidable mistakes might be measured and represented conveniently. The most vital errors having a place with this specific class are:

(a) Personal Errors:

These types of errors are exclusively caused due to personal mistakes or carelessness of the analyst. Careful working by the analyst can eliminate these type of errors.

Example: If analyst is wrongly calculating the weight of sodium hydroxide required to produce 0.1 N oxalic acid.

(b) Instrumental Errors:

Instrumental errors are due to defect in the equipment(s). These are invariably caused due to faulty and uncalibrated glasswares, apparatus and instruments. These errors can be removed by using good quality apparatus and calibrated glasswares, apparatus and instruments.

Example: Suppose a 5 ml pipette is used to take a sample solution for analysis but the pipette take out only 4.8 ml due to construction defect. Thus, an error of 0.2 ml will be introduced and this will always be on the negative side.

(c) Reagent Errors:

These errors are dependent on quality of the individual reagents. Many reagents or compounds are not in pure form, they contain impurities. Due to presence of impurity error occurs.

Example: If purity of the potassium hydrogen phthalate available in the laboratory is 90% pure and analyst is dissolving 204 g of that compound into 1000 ml distilled water to get the solution of 1 N solution. Then actual normality of the solution will be 0.9 N instead of 1 N; this 0.1 N error occurs due to reagent error.

(d) Additive or Constant Errors:

Sometimes the value of error is constant in a series determination and is independent of the amount of sample taken for analysis; these are termed as additive errors.

Example: In a titration, 0.1 ml extra titrant has to be added to see the colour change clearly at the end point. i.e. end point error is 0.1 ml. Therefore , if the standard value is 10 ml, the observed value is 10.1 ml.

If the sample taken for the titration is doubled, the standard value will be 20 ml, but in the titration value comes out to be 20.1 ml.

It should be noted that the error should remain the same. i.e. 0.1 ml.

(e) Proportional Errors:

In this type of error the magnitude of the error depends upon the sample size.

Example: In the titration of 10.0 ml of 0.1 N HCl, 10.0 ml (standard value) of 0.1 N NaOH solution should be required. But the NaOH used is impure so the observed value (volume of titrant) comes out to be 10.2 ml.

The error in the determination is 0.2 ml.

It means on doubling the sample the error is also doubled.

Hence, the error observed is proportional error.

(f) Errors in Method:

Any error occured during the method or selection of wrong method comes under this category.

Example: If any method involves chemical reaction which takes long time to complete and the method is carried out to the next step but the reaction is incomplete at that stage, then error in the method occurs.

2. Indeterminate/Random Errors

These errors are also known as non-systemic or accidental errors. The cause of a random error may or may not be known. Indeterminate errors are not one sided and they cannot be eliminated even when the analysis is done with the great care using high quality apparatus

and reagents. Random errors are due to causes over which the analyst has no control. When the same quantity is measured several times, different observed value will not be similar and this difference is called indeterminate error.

Example: A ball of 10 g is weighed on an analytical balance and we want to know its weight to the nearest of a gram i.e. whether it is 8, 9 or 10 replicate result will be obtained.

But if we want to know the weight of the ball upto the fourth place of decimal, the different weight readings will vary slightly from one another and also from the standard value of the weight of the ball. i.e. 10.0001, 10.0004, 10.0000, 10.0002 etc.

1.6.3 Methods of Minimizing Errors

Errors can be minimized by following the various techniques explained below:

Calibration of apparatus: By calibrating all the instruments, errors can be minimized and appropriate corrections are applied to the original measurements.

Control determination: standard substance is used in experiment in identical experimental condition to minimize the errors.

Blank determination: By omitting sample, a determination is carried out in identical condition to minimize the errors occured due to impurities present in reagent.

Independent method of analysis: It is carried out to maintain accuracy of the result e.g. Iron (III) is first determined gravimetrically by precipitation method as iron (III) hydroxide and then determined titrimetrically by reduction to the iron (II) state.

Parallel determination: Instead of single determination, duplicate or triplicate determination is carried out to minimize the possibilities of accidental errors.

Standard addition: This method is generally applied to physico-chemical procedures such as polarography and spectrophotometry.

Internal standards: It is used in spectroscopic and chromatographic determination.

Amplification methods: It is used when a very small amount of material is to be measured which is beyond the limit of the apparatus.

Isotopic dilution: It is used for the compound containing radioactive isotope.

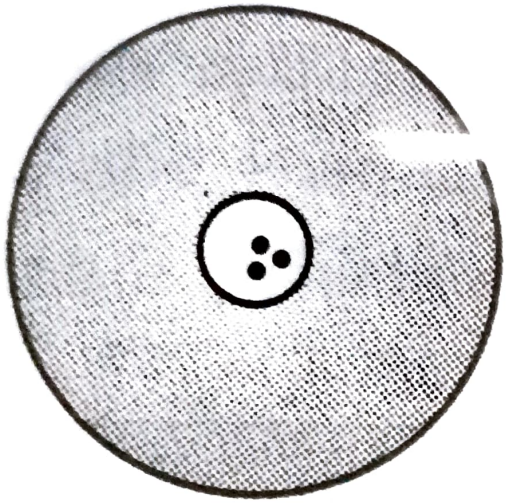
1.6.4 Accuracy and Precision

Accuracy:

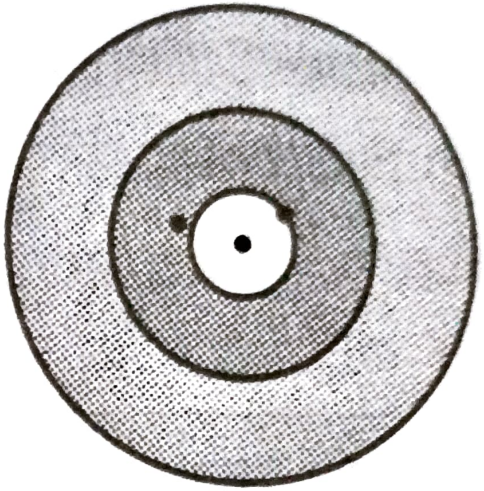
Accuracy is 'the degree of agreement between the measured value and the true value'. An absolute true value is seldom known. So, the term accuracy refers to how near the observed value is to true or standard value.

Precision:

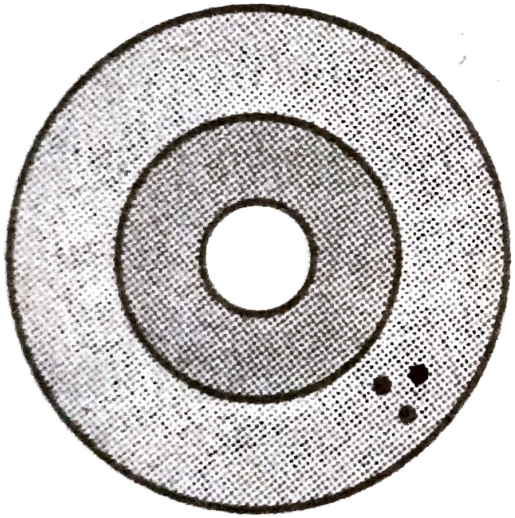
Precision is defined as 'the degree of agreement between replicate measurements of the same quantity'. It is the repeatability of a result. The precision may be expressed as the standard value. So, the term precision refers to nearness between several measurements of the same quantity.



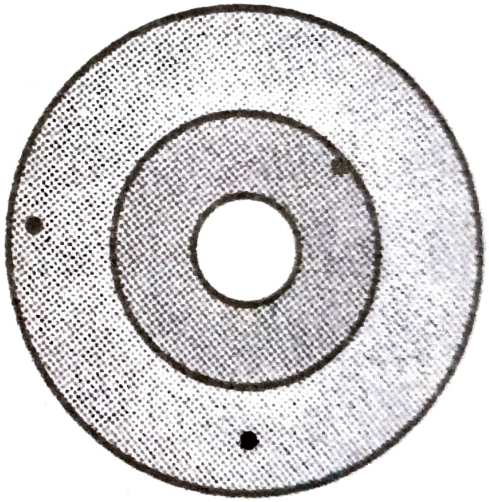
(a) Accuracy and Precision



(b) Accuracy without Precision



(c) Precision without Accuracy



(d) No Precision and No Accuracy

Fig. 1.7: Accuracy and Precision

1.6.5 Significant Figures

The significant figures of a number are those digits that carry meaning contributing to its measurement resolution. This includes all digits except –

- All leading zeros (A leading zero is any 0 digit that comes before the first non-zero digit in a number).
- Trailing zeros when they are merely placeholders to indicate the scale of the number; and
- Spurious digits introduced, for example, by calculations carried out to greater precision than that of the original data, or measurements reported to a greater precision than the equipment supports.

Specifically, the rules for identifying significant figures when writing or interpreting numbers are as follows:

- All non-zero digits are considered significant. For example, 42 has two significant figures (4 and 2), while 142.35 has five significant figures (1, 4, 2, 3 and 5).
- Zeros appearing anywhere between two non-zero digits are significant. Example: 405.7209 have seven significant figures: 4, 0, 5, 7, 2, 0 and 9.
- Leading zeros are not significant. For example, 0.00034 has two significant figures: 3 and 4.
- Trailing zeros in a number containing a decimal point are significant. For example, 22.5200 has six significant figures: 2, 2, 5, 2, 0 and 0. The number 0.000397800 still has only six significant figures (the zeros before the 3 are not significant). In addition, 260.00 have five significant figures since it has three trailing zeros. This convention clarifies the precision of such numbers; for example, if a measurement precise to four decimal places (0.0001) is given as 15.87 then it might be understood that only two decimal places of

precision are available. Stating the result as 15.8700 makes clear that it is precise to four decimal places (in this case, six significant figures).

- The significance of trailing zeros in a number not containing a decimal point can be indistinct.

1.7 PHARMACOPOEIA

Pharmacopoeia is an official publication containing methodology to identify the medicinal compounds. It is published by the government authority, medical or pharmaceutical society. Descriptions of the compounds are given in the form of monographs in the pharmacopoeia. It is a reference work for pharmaceutical drug specifications.

The term derives from Ancient Greek word *pharmakopoiia* which means "drug-making", from *pharmakon* or drug.

List of National and Supranational Pharmacopoeias:

- **Brazilian Pharmacopoeia:** This pharmacopoeia is published by Brazilian Health Surveillance Agency in Brazil.
- **British Pharmaceutical Codex:** The British Pharmaceutical Codex (BPC) was first published in 1907, to supplement the British Pharmacopoeia which although extensive, did not cover all the medicinal items that a pharmacist might require in daily work.
- **British Pharmacopoeia:** The British Pharmacopoeia (BP) is the national pharmacopoeia of the United Kingdom. It is an annual published collection of quality standards for UK medicinal substances. It is used by individuals and organizations involved in pharmaceutical research, development, manufacture and testing.
- **Czech Pharmacopoeia:** It is essential pharmaceutical work prescriptive character binding on the territory of the Czech Republic. Contributes to ensuring the safety, efficacy and quality of pharmaceuticals .
- **Indian Pharmacopoeia:** Indian Pharmacopoeia Commission (IPC) 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. The set of standards are published under the title Indian Pharmacopoeia (IP) which has been modelled over and historically follows from the British Pharmacopoeia.
- **International Pharmacopoeia:** It is a pharmacopoeia issued by the World Health Organization as a recommendation, with the aim to achieve a wide global uniformity of quality specifications for selected pharmaceutical drugs, excipients and dosage forms.
- **Japanese Pharmacopoeia:** It is the official Pharmacopoeia of Japan. It is published by the *Pharmaceuticals and Medical Devices Agency* under the authority of the Ministry of Health, Labour and Welfare.

- **Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia):** It is compiled by the Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China. It is an official compendium of drugs, covering Traditional Chinese and western medicines, which includes information on the standards of purity, description, test, dosage, precaution, storage and the strength for each drug.
- **The European Pharmacopoeia:** It is a major regional pharmacopoeia which provides common quality standards throughout the pharmaceutical industry in Europe to control the quality of medicines, and the substances used to manufacture them.
- **The United States Pharmacopeia:** It is a pharmacopeia (compendium of drug information) for the United States published annually by the United States Pharmacopeial Convention (usually also called the USP), a non-profit organization that owns the trademark and copyright.

1.8 SOURCES OF IMPURITIES IN MEDICINAL AGENTS

Impurities are representative of admixture of foreign material in any compound. Pure chemical compounds are free from any foreign material.

In chemical analysis impurity plays a significance role and affects the results; so sources of impurities should be known to the analyst to determine the magnitude of the impurity present in the compound. Various pharmacopoeias prescribed guidelines to determine the amount of impurities as well as standard permissible limit of impurities.

Various sources of impurities in medicinal agents are possible; i.e.

- **Raw Materials:** Raw materials employed in the manufacturing of the pharmaceutical substance or drugs are major sources of impurities. Rock salt used for the preparation of sodium chloride is contaminated with small amounts of calcium and magnesium chlorides.
- **Manufacturing Process:** Process or method of manufacture may introduce new impurities into the final product arising due to contamination by reagents, catalysts and solvents employed at various stages of the manufacturing process.
- **Instability of the Product:** Impurities can also arise during storage because of chemical instability of the pharmaceutical substance. Many pharmaceutically important substances undergo chemical decomposition when storage conditions are inadequate. Pharmaceuticals may undergo changes in physical properties during storage. There can be changes in crystal size and shape, sedimentation, agglomeration and caking of the suspended particles.
- **Reaction with Packaging Materials:** The possibility of reaction between the container material and the contents cannot be ruled out as it constituents a safety hazard.
- **Temperature:** The rate of chemical decomposition and physical changes of stored products depends upon the temperature, because it may change the composition of the compound and leads impurity.

1.9 LIMIT TESTS

Limit tests are quantitative or semi-quantitative tests especially set forward to identify and control perpetually little amounts of impurities that should be available in a pharmaceutical substance. Clearly, the measure of any single impurity present in an official substance is typically small, and in this manner, the ordinary visible reaction to any test for that contamination is very little. Henceforth, it is vital and imperative to plan the individual test in such a way in order to dodge possible errors in the hands of analysts. It might be accomplished by thinking about the accompanying three cardinal components, to be specific.

- *Specificity of the Tests:* A test utilized as an limit test ought to suggest some kind of particular response with the trace impurity. It has been observed that a less specific test which restricts various possible impurities rather directly has a positive edge over the highly specific tests.
- *Sensitivity:* The degree of sensitivity stipulated in a limit test fluctuates broadly according to the standard set by pharmacopoeia. The sensitivity is administered by various variable components having a typical goal to yield reproducible outcomes, for example Gravimetric Analysis, Colour Tests,
- *Personal Errors:* Personal error must be avoided by the analyst at any stage of the experiment.

1. Limit Test for Chloride:

Aim: To carry out limit test for chloride.

Requirements: Nessler cylinder, dilute nitric acid, silver nitrate solution, distilled water, glass rod.

Principle:

The limit test for chloride is based on its precipitation phenomenon. Chloride is precipitated with silver nitrate in the presence of dilute HNO_3 and comparing the turbidity produced due to the formation of AgCl with a standard solution of known quantity of chloride ions.

$$NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$$

Preparation of Reagents:

Preparation of 10% w/w of HNO_3:

Percent purity of concentrated nitric acid is 70% and density is 1.66. 10 g nitric acid is required to prepare 100 ml 10% w/w nitric acid (Density of the water is 1.00). So, add slowly 8.6 ml concentrated nitric acid in the distilled water of around 50 ml and make up the final volume upto 100 ml in a volumetric flask.

Preparation of 5% w/w of $AgNO_3$:

Dissolve 5 g of silver nitrate in 50 ml of distilled water and make up the final volume upto 100 ml in a volumetric flask.

Preparation of 25 ppm chloride solution:

Dilute 5 volumes of a 0.0824 percent w/v solution of *sodium chloride* to 100 volumes with distilled *water*.

Experimental Methodology:

1. Dissolve the specific weighed quantity of the sample substance in distilled water or directly take the liquid solution into a nessler cylinder.
2. Take 10 ml of 25 ppm chloride solution in another nesseler cylinder.
3. Add 10 ml of dilute nitric acid and dilute the solution upto 50 ml with distilled water, and finally add 1 ml of 0.1 M silver nitrate solution in both the nessler cylinders.
4. Observe the opalescence of both by viewing transversely against a black background.
5. If opalescence of sample is less intense than the standard chloride solution, sample passes the limit test for chloride.

Result:

The given sample passed/failed the limit test for chloride.

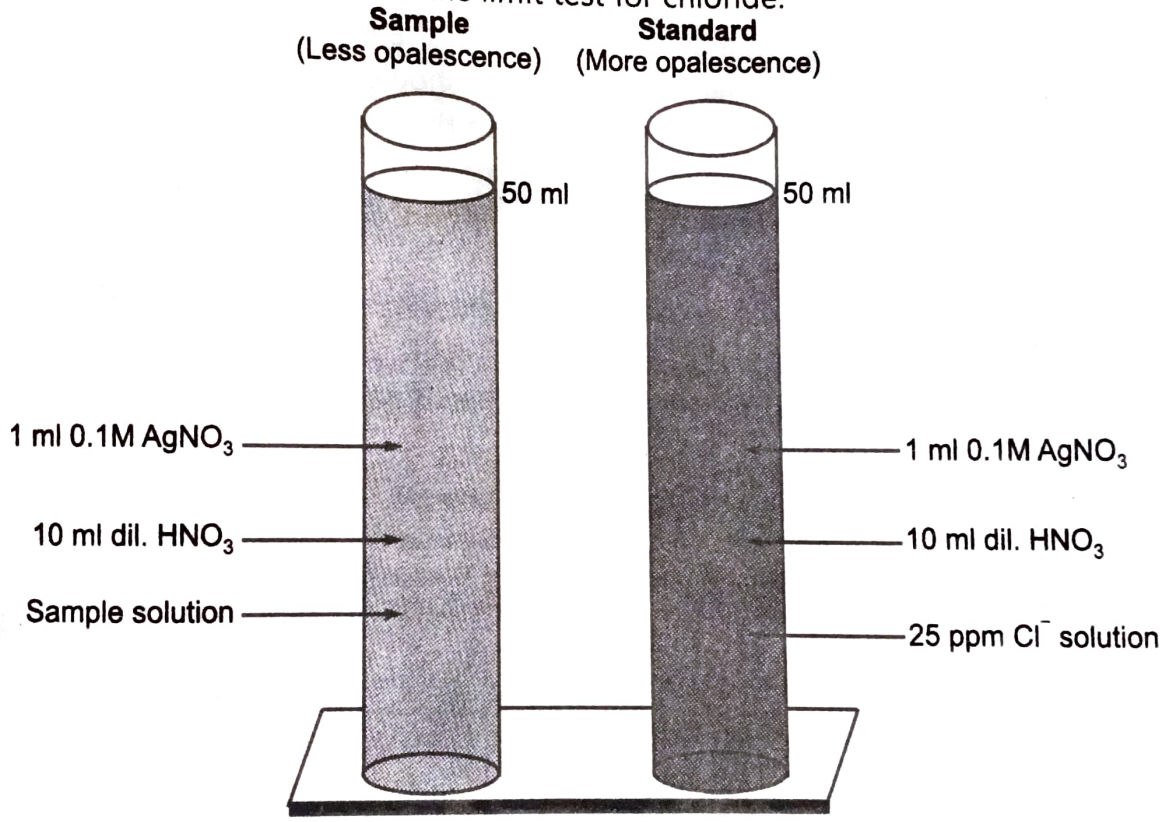


Fig. 1.8: Limit test for chloride

2. Limit Test for Arsenic:

Aim: To carry out limit test for arsenic.

Requirements: Potassium iodide, zinc, arsenic trioxide, sodium hydroxide, distilled water, arsenic limit test apparatus.

Principle:

The official process is a development of the Gutzeit Test. All arsenic present is duly converted into arsine gas (AsH_3) by reduction with Zn and HCl. Further, it depends upon the fact that when arsine comes into contact with dry paper permeated with mercuric chloride it produces a yellow strain, the intensity of which is directly proportional to the quantity of arsenic present. The various chemical reactions involved may be expressed by the equations:

$$Zn + 2\,HCl \longrightarrow ZnCl_2 + 2(H)$$
$$2As + 6(H) \longrightarrow 2AsH_3 \uparrow$$
$$HgCl_2 + AsH_3 \longrightarrow \underbrace{HgCl_2 \cdot AsH_3}_{\text{Yellow complex}}$$

Preparation of Reagents:

Preparation of 1 M KI solution:

Dissolve 16.6 g of KI in 50 ml of distilled water and make up the final volume upto 100 ml in a volumetric flask.

Preparation of arsenic standard solution (10 ppm):

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.

Experimental Methodology:

1. Take the test solution into the bottle or conical flask, add 5 ml of 1 M KI and 10 g of Zn. Immediately assemble the apparatus and immerse the flask in a water bath at constant temperature so that uniform evaluation of the gas should be maintained.
2. Take arsenic standard solution (10 ppm) 1 ml into the bottle or conical flask, add 5 ml of 1 M KI and 10 g of Zn. Immediately assemble the apparatus and immerse the flask in a water bath at constant temperature so that uniform evaluation of the gas should be maintained.
3. Observe the presence of stains in the mercuric chloride paper and compare the sample and standard.
4. If intensity of the stain is less in the test sample then it passes the limit test for arsenic.

Result:

The given sample passed/failed the limit test for arsenic.

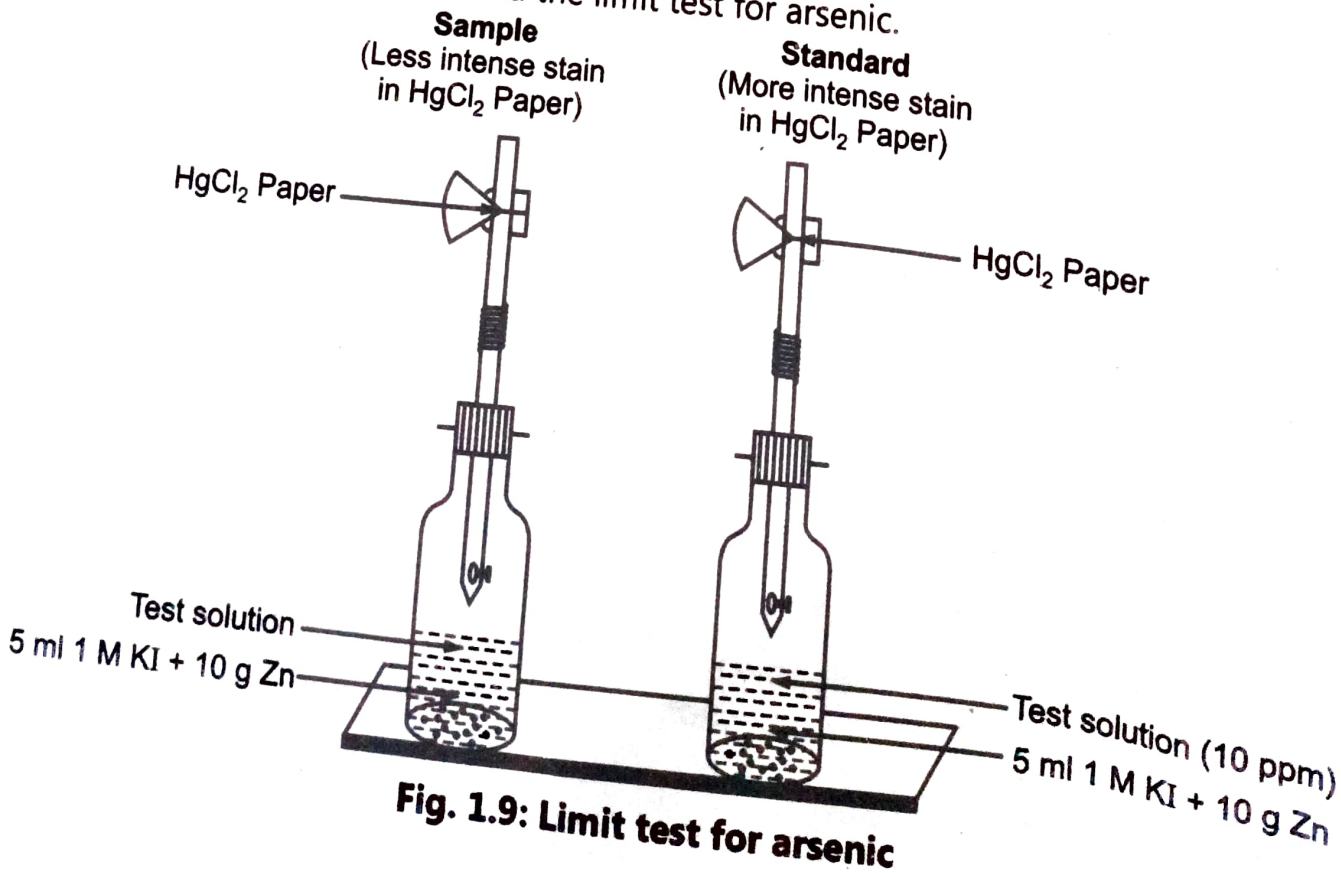


Fig. 1.9: Limit test for arsenic

3. Limit Test for Sulphate:

Aim: To carry out limit test for sulphate.

Requirements: Barium chloride, glacial acetic acid, potassium sulphate, ethanol, distilled water, nessler cylinder.

Principle:

The limit test for sulphates is based upon its precipitation as barium sulphate in the presence of barium chloride, hydrochloric acid and barium sulphate. In this reaction, hydrochloric acid exerts its common ion effect whereas traces of $BaSO_4$ aids in the rapid and complete precipitation by seeding. Thus, the opalescence caused by the sample is compared immediately with a standard turbidity produced with a known amount of the SO^{2-} ion.

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

Preparation of Reagents:

Preparation of 25.0 percent w/v $BaCl_2$ solution:

Take 25 g of $BaCl_2$ and dissolve in 50 ml of distilled water and make up the final volume upto 100 ml.

Preparation of 5 M acetic acid:

Take 28.73 ml of glacial acetic acid and dissolve in 50 ml of distilled water and finally make up the volume upto 100 ml in a volumetric flask.

Preparation of 10 ppm SO_4^{2-} ethanolic solution:

Dilute 1 volume of a 0.181 percent w/v solution of potassium sulphate in ethanol (30%) to 100 volumes with the same solvent.

Preparation of 10 ppm SO_4^{2-} solution:

Dilute 1 volume of a 0.181 percent w/v solution of potassium sulphate in distilled water to 100 volumes with the same solvent.

Experimental Methodology:

1. Take 1.0 ml of a 25.0% w/v solution of $BaCl_2$ in a nessler cylinder, add 1.5 ml of ethanolic standard sulphate solution (10 ppm) mixed properly and allow to stand for 1 minute. Add 15 ml of the sample solution (prepared as directed in the monograph or a solution of the specified quantity of the substance under examination in 15 ml of water and 0.15 ml of 5 M acetic acid) to the nessler cylinder; finally add sufficient distilled water to produce 50 ml solution, stir immediately with a glass rod and allow to stand for 5 minutes.
2. Prepare a standard sample solution with same procedure by using 15 ml standard sulphate solution (10 ppm) in the place of 15 ml sample solution.
3. Compare both the nessler cylinder transversely against a black background. Any opalescence produced should not be more intense than the standard.

Result:

The given sample passed/failed the limit test for sulphate.

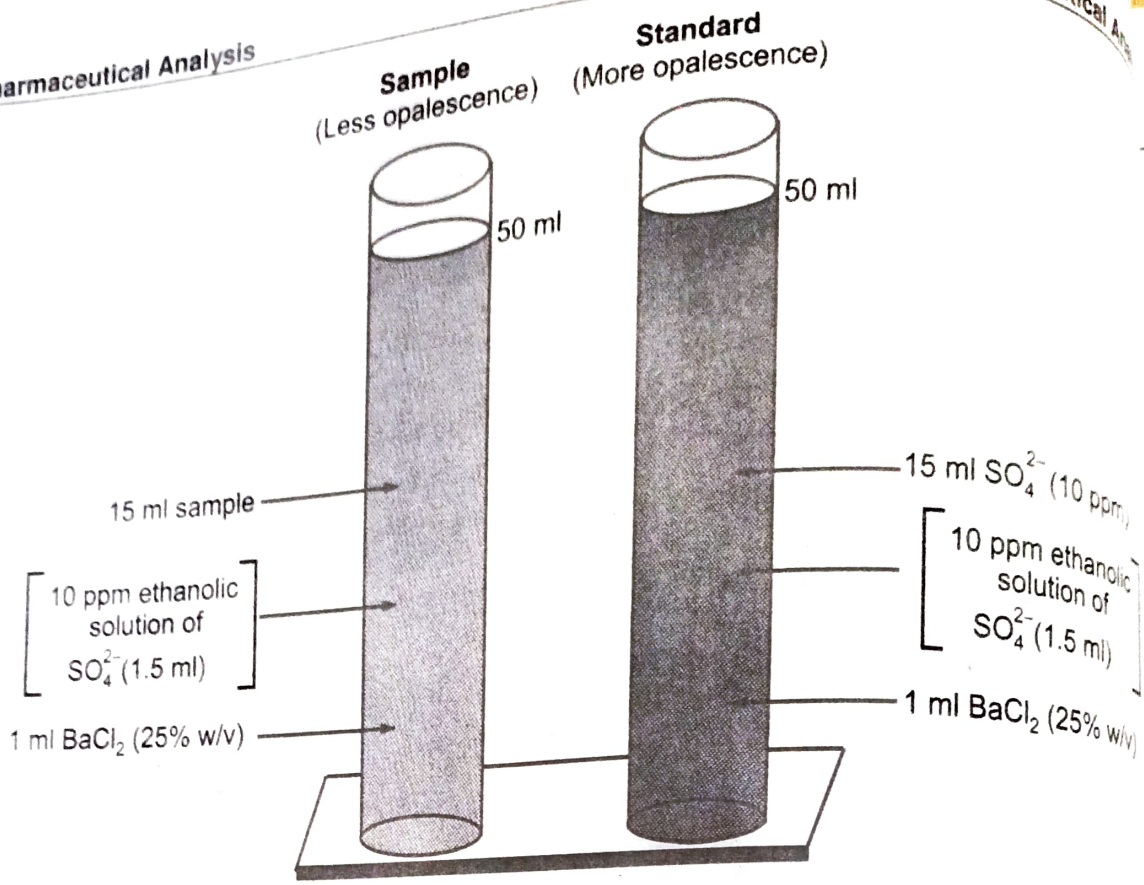


Fig. 1.10: Limit test for sulphate

4. Limit Test for Iron:

Aim: To carry out limit test for iron.

Requirements: Ferric ammonium sulphate, sulphuric acid, citric acid, thioglycollic a ammonia solution, distilled water, nessler cylinder.

Principle:

The limit test for Iron is based on the reaction between iron and thioglycollic acid i ammonium citrate buffer solution to give a purple colour, which is consequently compa with the standard colour obtained with a known amount of iron (0.04 mg of Fe). Ferro thioglycollate is a co-ordination compound that attributes the purple colour; basic thioglycollic acid converts the entire Fe^{3+} into Fe^{2+}.

$$2Fe^{3+} + 2HS \cdot CH_2 \cdot COOH \longrightarrow 2Fe^{2+} + HOOC \cdot CH_2SSCH_2 \cdot COOH + 2H^+$$

$$Fe^{2+} + 2HS \cdot CH_2 \cdot COOH \longrightarrow \text{Ferrous thioglycollate} + 2H^+$$

Preparation of Reagents:

Preparation of citric acid solution (20% w/v):

Dissolve 20 g of citric acid into 60-70 ml of distilled water and make the final volu upto 100 ml in a volumetric flask.

Preparation of 20 ppm iron standard solution:

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. The prepared solution contains iron in ferric state.

Experimental Methodology:

1. Dissolve the specific amount of test sample in distilled water and transfer into a nessler cylinder. Add 2 ml of iron free citric acid solution (20% w/v) and 0.1ml thioglycollic acid. Mix the solution and make alkaline with iron free ammonia solution and dilute upto 50 ml by distilled water.
2. Take iron standard solution (20 ppm), add 2 ml of iron free citric acid solution (20% w/v) and 0.1 ml thioglycollic acid. Mix the solution and make alkaline with iron free ammonia solution and dilute upto 50 ml by distilled water.
3. Compare both the nessler cylinders for intensity of colour, if test sample containing solution is less intense then it passes the limit test for iron.

Result:

The given sample passed/failed the limit test for iron.

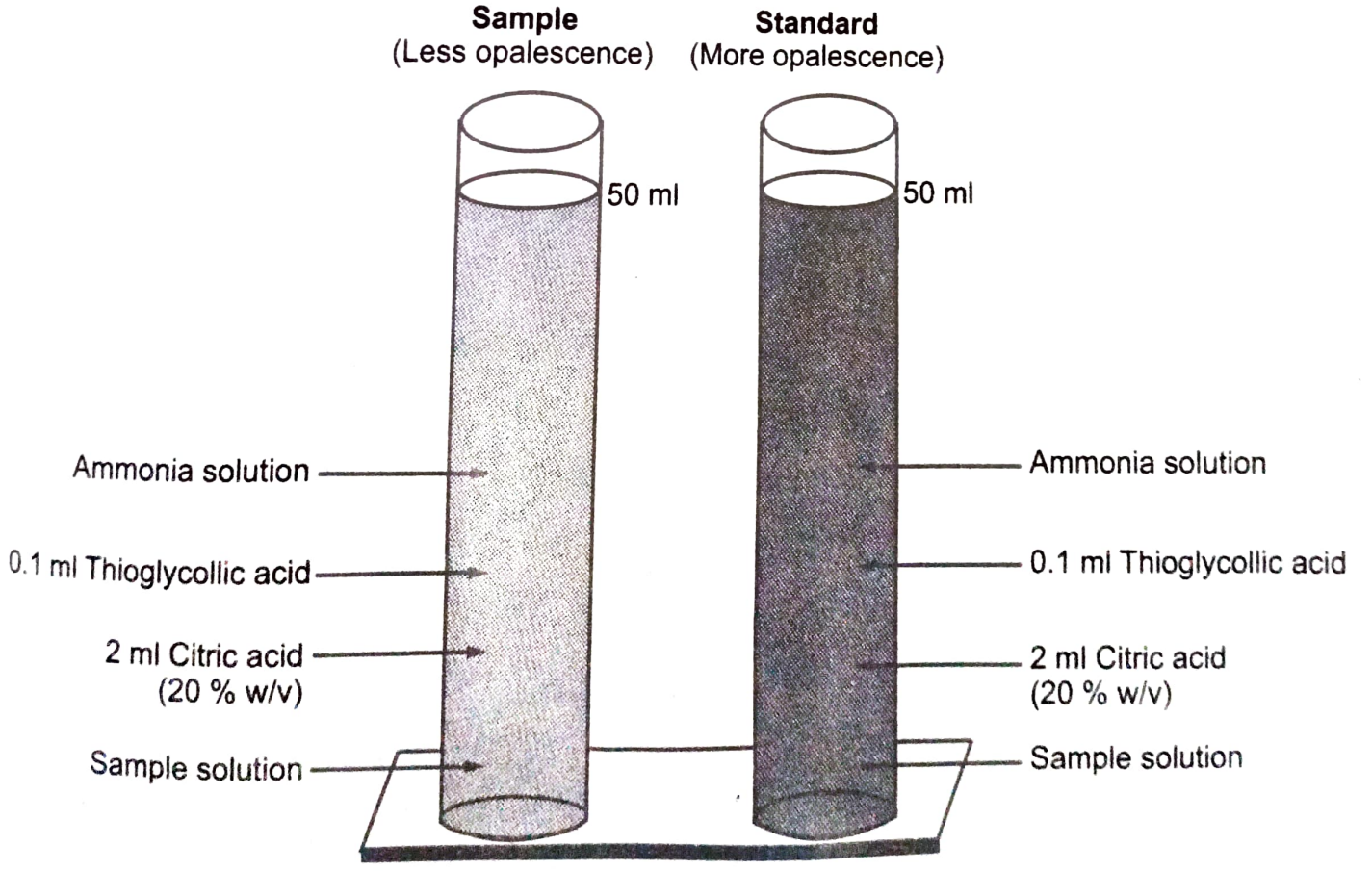


Fig. 1.11: Limit test for iron