Pharmaceutical Analysis

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Pharmaceutical Analysis

Definition:- **Pharmaceutical Analysis** is the process of evaluating and determining the **quality, purity, identity, potency, and stability** of drugs and pharmaceutical formulations using various **chemical, physical, and biological methods**.

Scope of Pharmaceutical Analysis

1. Identification of drugs and raw materials
2. Quantitative estimation of drug substances
3. Purity and impurity profiling
4. Stability testing of pharmaceutical products
5. Quality control of raw materials and finished products
6. Quality assurance in manufacturing processes
7. Analytical method development and validation
8. Bioanalytical testing in biological fluids
9. Regulatory compliance and documentation
10. Detection of counterfeit and substandard drugs

Study of Analysis

I. **Manual Method of Analysis:**
As organoleptic properties like taste, odour, texture, colour, and appearance can be observed through sense organs.

II. **Chemical Method of Analysis:**
Different kinds of chemical methods are there to analyse our sample.
For example, different types of titrations come under chemical methods of analysis as it involves chemical reactions by reagents.

III. **Instrumental Method of Analysis:**
This involves various instrumental methods to analyse any kind of sample by different instruments like UV spectroscopy, HPLC, NMR, IR spectroscopy etc.

Different Techniques of Analysis

Studying various **techniques of analysis** is essential in pharmaceutical, chemical, environmental, and biological sciences for **accurate identification, quantification, and evaluation** of substances.

Techniques of Analysis

1. Chemical Methods
    - I. Volumetric or titrimetric methods
    - II. Gravimetric methods
    - III. Gasometric methods
2. Electrical Methods
3. Instrumental Methods
4. Chromatographic and Electrophoretic Methods
5. Biological and Microbiological Methods

1. Chemical Methods

I) Volumetric or titrimetric methods

☐ **Volumetric analysis** (or titrimetric analysis) is a quantitative method in which the amount of a substance is determined by measuring the volume of a standard solution (titrant) required to complete a chemical reaction.

☐ **Basic Principle:**

A known volume of the sample solution reacts with a titrant of known concentration until the **equivalence point** is reached. The **end point** is detected using an **indicator** or instrumentally.

☐ **Key Components:**

i. **Titrant** – A solution of known concentration.
ii. **Analyte** – The substance whose concentration is to be determined.
iii. **Indicator** – A chemical that changes color at the end point.
iv. **Burette** – For accurate delivery of titrant.
v. **Equivalence Point** – Point at which chemically equivalent amounts react.
vi. **End Point** – The observed point where titration is stopped (usually by indicator color change).

Types of Volumetric analysis or titrimetric analysis

S.No.	Type of Titration	Principle	Common Reagents	Example
1.	Acid-Base Titration	Based on neutralization between acid and base	HCl, NaOH, Phenolphthalein	NaOH vs HCl
2.	Redox Titration	Based on oxidation-reduction reaction	$KMnO_4$, $K_2Cr_2O_7$, Iodine	$KMnO_4$ vs Fe^{2+}
3.	Complexometric Titration	Based on formation of a stable complex between metal ion and ligand	EDTA, Eriochrome Black T	EDTA vs Ca^{2+}/Mg^{2+}
4.	Precipitation Titration	Based on formation of a precipitate during titration	$AgNO_3$, K_2CrO_4	$AgNO_3$ vs Cl^- (Mohr's method)
5.	Non-aqueous Titration	Used for substances insoluble or unstable in water	Perchloric acid in acetic acid	Assay of weak bases in non-aqueous media

II) Gravimetric analysis

- ❑ **Definition**:- **Gravimetric analysis** is a **quantitative analytical technique** in which the **amount of an analyte** is determined by **measuring mass**. The analyte is converted into a **pure, stable, and weighable solid** form.
- ❑ **Basic Principle:-** The analyte is isolated in a measurable solid form, either by **precipitation** or **volatilization**, and then **weighed accurately** to determine its quantity.
- ❑ **Steps in Precipitation Gravimetric Analysis:**
  - **i.** **Preparation of Solution** – Dissolve sample completely.
  - **ii.** **Precipitation** – Add reagent to form an insoluble precipitate.
  - **iii.** **Digestion** – Allow precipitate to form large, pure crystals.
  - **iv.** **Filtration** – Separate the precipitate using filter paper.
  - **v.** **Washing** – Remove impurities from the precipitate.
  - **vi.** **Drying/Ignition** – Convert to a stable form and remove moisture.
  - **vii.** **Weighing** – Accurately weigh the dried precipitate.
  - **viii.** **Calculation** – Determine amount of analyte based on stoichiometry.

Types of Gravimetric Methods

S.No.	Type	Description	Example
1.	Precipitation Method	Analyte is precipitated as an insoluble compound and weighed.	Determination of chloride as AgCl.
2.	Volatilization Method	Analyte is converted into a volatile form and collected or lost during heating.	Determination of water by loss on drying.
3.	Electrogravimetry	Analyte is deposited on an electrode and weighed.	Determination of copper by electrolysis.
4.	Thermogravimetry (TGA)	Mass change is recorded as a function of temperature.	Analysis of thermal stability and composition.

III) Gasometric analysis

Definition:- **Gasometric analysis** is a **quantitative analytical technique** in which the **volume of a gas** (either **evolved** or **absorbed**) during a chemical reaction is **measured** to determine the amount of analyte present.

Basic Principle:-The amount of substance is determined based on the **volume of gas** involved in a **chemical reaction** under controlled **temperature and pressure**.

Types of Gasometric Methods:

Method	Gas Involved	Example
Gas Evolution	Gas is released and measured	Determination of carbonate by measuring CO_2
Gas Absorption	Gas is absorbed by a solution	Estimation of oxygen using alkaline pyrogallol
Gas Collection	Gas is collected over water or mercury	Hydrogen gas from reaction of acid with metal

2. Electrical Methods

Definition:-Electrical methods of analysis involve the measurement of **electrical properties** such as **conductance**, **potential**, **current**, or **charge** to determine the **composition or concentration** of substances in a sample.

Types of Electrical Methods

S.No.	Method	Principle	Measured Quantity	Applications
1.	Conductometry	Based on change in conductance of solution during titration	Conductance (G)	Acid-base titration, salt analysis
2.	Potentiometry	Measures potential difference between electrodes without current	Electrode potential (E)	pH measurement, redox titration, ion-selective analysis
3.	Amperometry	Current is measured at a fixed potential	Current (I)	Oxygen, glucose sensing
4.	Coulometry	Total charge passed during electrochemical reaction is measured	Charge (Q)	Trace analysis, Karl Fischer titration (moisture)
5.	Polarography	Current is measured while potential is varied (using mercury electrode)	Current vs. Voltage	Analysis of metal ions, vitamins
6.	Voltammetry	Measures current with applied varying voltage using different electrodes	Current (I)	Organic/inorganic compound analysis
7.	Electrogravimetry	Analyte is deposited on an electrode and weighed	Mass via current	Metal ion determination (e.g., Cu, Ni)

3. Instrumental Methods

Definition:-Instrumental methods of analysis are **analytical techniques** that use **instruments or devices** to measure physical or chemical properties of a substance in order to determine its **qualitative** or **quantitative composition**.

Types of Instrumental Methods

Category	Method	Measured Property	Examples
Spectroscopic Methods	UV-Visible Spectroscopy	Absorbance of UV/Vis light	Drug purity, concentration
	Infrared (IR) Spectroscopy	Molecular vibrations	Functional group identification
	Atomic Absorption Spectroscopy (AAS)	Light absorbed by free atoms	Metal ion analysis (e.g., Ca^{2+}, Zn^{2+})
	Flame Photometry	Light emitted by excited atoms	Na^+, K^+ in biological fluids
	Fluorimetry	Fluorescence emitted	Vitamin B1, drugs in low concentration
Chromatographic Methods	Thin Layer Chromatography (TLC)	Separation based on polarity	Drug identification, purity check
	Gas Chromatography (GC)	Separation of volatile compounds	Analysis of essential oils, solvents
	High Performance Liquid Chromatography (HPLC)	Separation in liquid phase	Drug formulation analysis
Electrochemical Methods	Potentiometry	Electrode potential	pH, redox titrations
	Conductometry	Conductance	Acid-base titrations
	Voltammetry	Current response to voltage	Trace metal ions

4. Biological and Microbiological Methods

Definition:-Biological and Microbiological methods involve the use of **living organisms**, **cells**, **enzymes**, or **microorganisms** to measure the **activity**, **potency**, or **concentration** of chemical substances, especially **drugs**, **antibiotics**, and **toxins**.

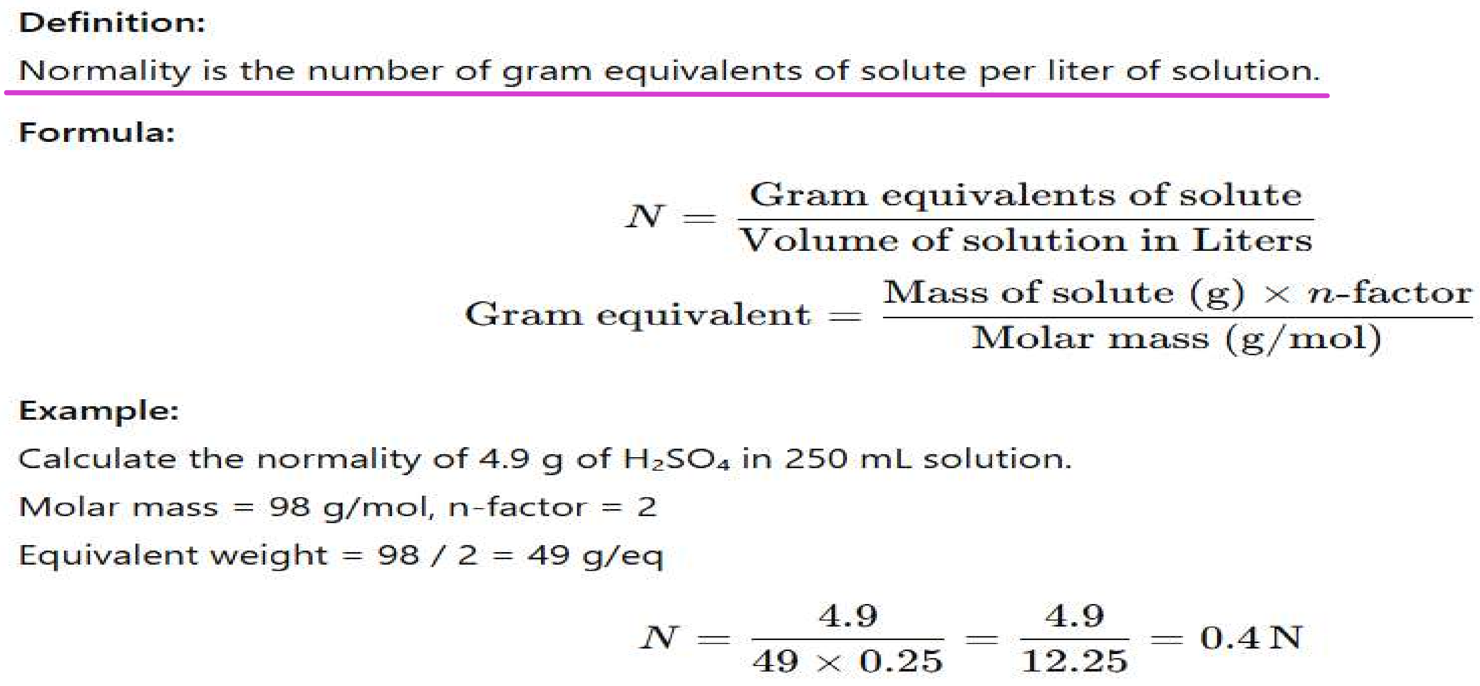
Types of Biological and Microbiological Methods

S.No.	Method	Basis	Example Application
1.	Bioassay	Measuring drug potency using biological response	Assay of insulin, heparin, digitalis
2.	Microbiological Assay	Measuring antimicrobial activity using microorganisms	Penicillin, streptomycin, tetracycline assay
3.	Enzymatic Methods	Use of enzymes to catalyze reactions for quantification	Glucose estimation using glucose oxidase
4.	Tissue Culture Assay	Measuring cell growth or response in culture	Cytotoxicity testing of anticancer drugs
5.	Immunoassay (ELISA, RIA)	Antigen-antibody interactions for detection	Hormone level detection, virus detection (e.g., HIV)

Methods of Expressing Concentration in Analysis

In pharmaceutical and chemical analysis, **concentration** refers to the amount of **solute** present in a given quantity of **solvent or solution**. It is essential for preparing standard solutions and analyzing samples accurately.

1. Normality (N)



Definition:

Normality is the number of gram equivalents of solute per liter of solution.

Formula:

$$N = \frac{\text{Gram equivalents of solute}}{\text{Volume of solution in Liters}}$$

$$\text{Gram equivalent} = \frac{\text{Mass of solute (g)} \times n\text{-factor}}{\text{Molar mass (g/mol)}}$$

Example:

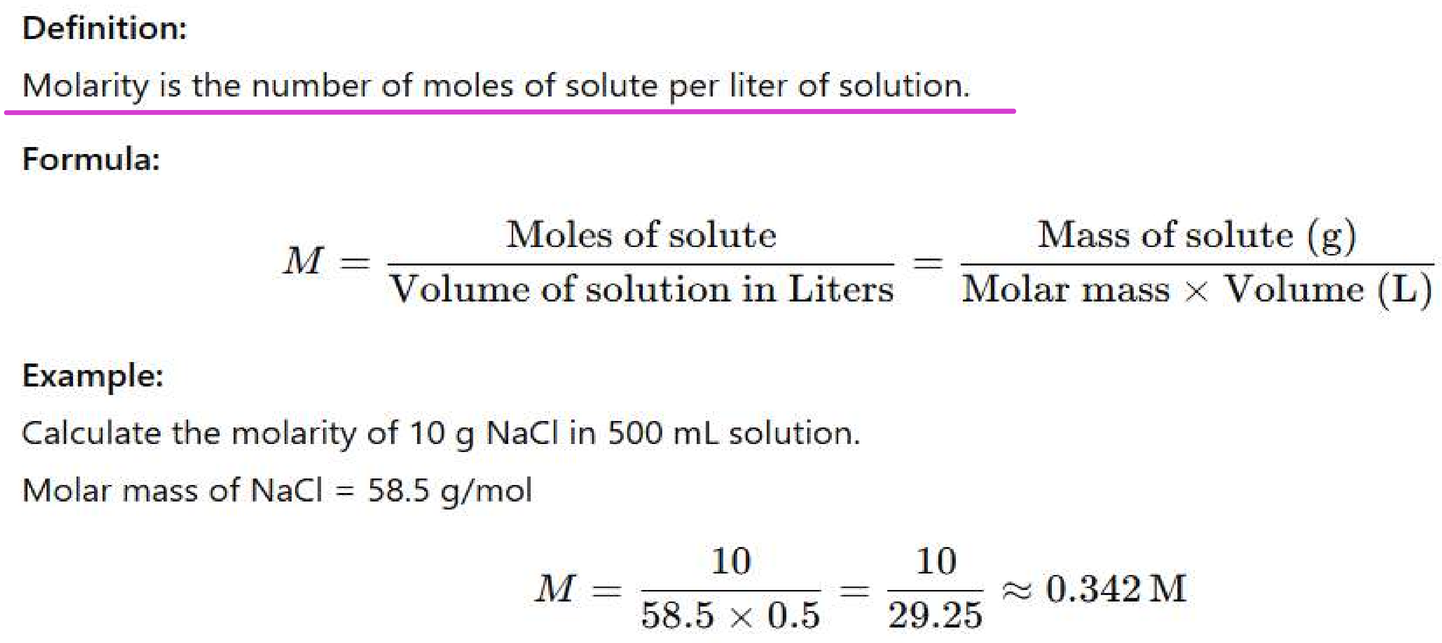
Calculate the normality of 4.9 g of H_2SO_4 in 250 mL solution.

Molar mass = 98 g/mol, n-factor = 2

Equivalent weight = 98 / 2 = 49 g/eq

$$N = \frac{4.9}{49 \times 0.25} = \frac{4.9}{12.25} = 0.4\,\text{N}$$

2. Molarity (M)



Definition:

Molarity is the number of moles of solute per liter of solution.

Formula:

$$M = \frac{\text{Moles of solute}}{\text{Volume of solution in Liters}} = \frac{\text{Mass of solute (g)}}{\text{Molar mass} \times \text{Volume (L)}}$$

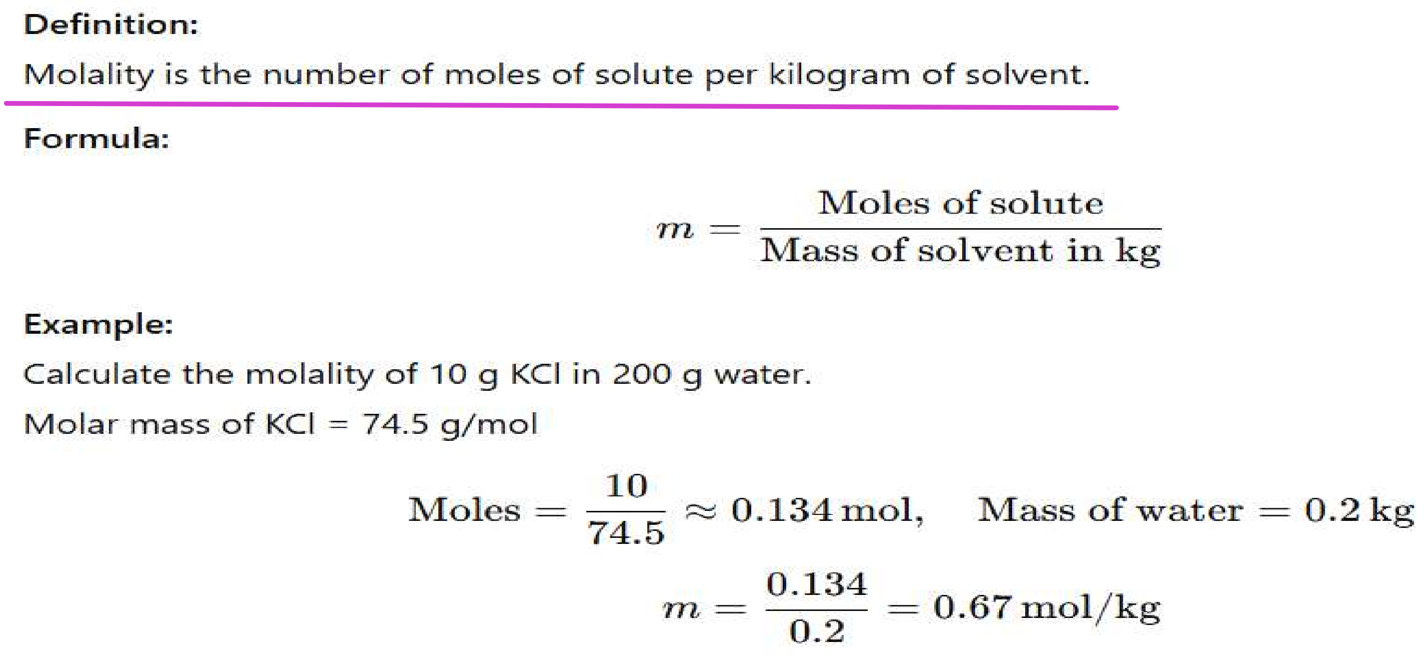
Example:

Calculate the molarity of 10 g NaCl in 500 mL solution.

Molar mass of NaCl = 58.5 g/mol

$$M = \frac{10}{58.5 \times 0.5} = \frac{10}{29.25} \approx 0.342\,\mathrm{M}$$

3. Molality (m)



Definition:

Molality is the number of moles of solute per kilogram of solvent.

Formula:

$$m = \frac{\text{Moles of solute}}{\text{Mass of solvent in kg}}$$

Example:

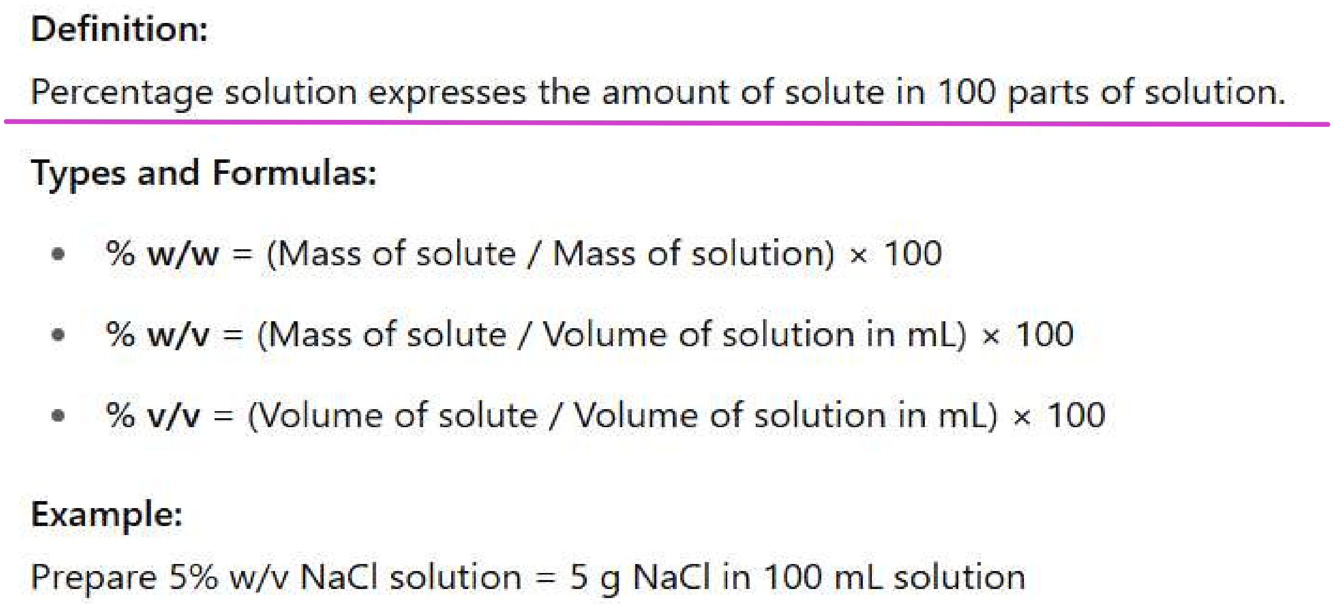
Calculate the molality of 10 g KCl in 200 g water.

Molar mass of KCl = 74.5 g/mol

$$\text{Moles} = \frac{10}{74.5} \approx 0.134\,\text{mol}, \quad \text{Mass of water} = 0.2\,\text{kg}$$

$$m = \frac{0.134}{0.2} = 0.67\,\text{mol/kg}$$

4. Percent Solution



Definition:

Percentage solution expresses the amount of solute in 100 parts of solution.

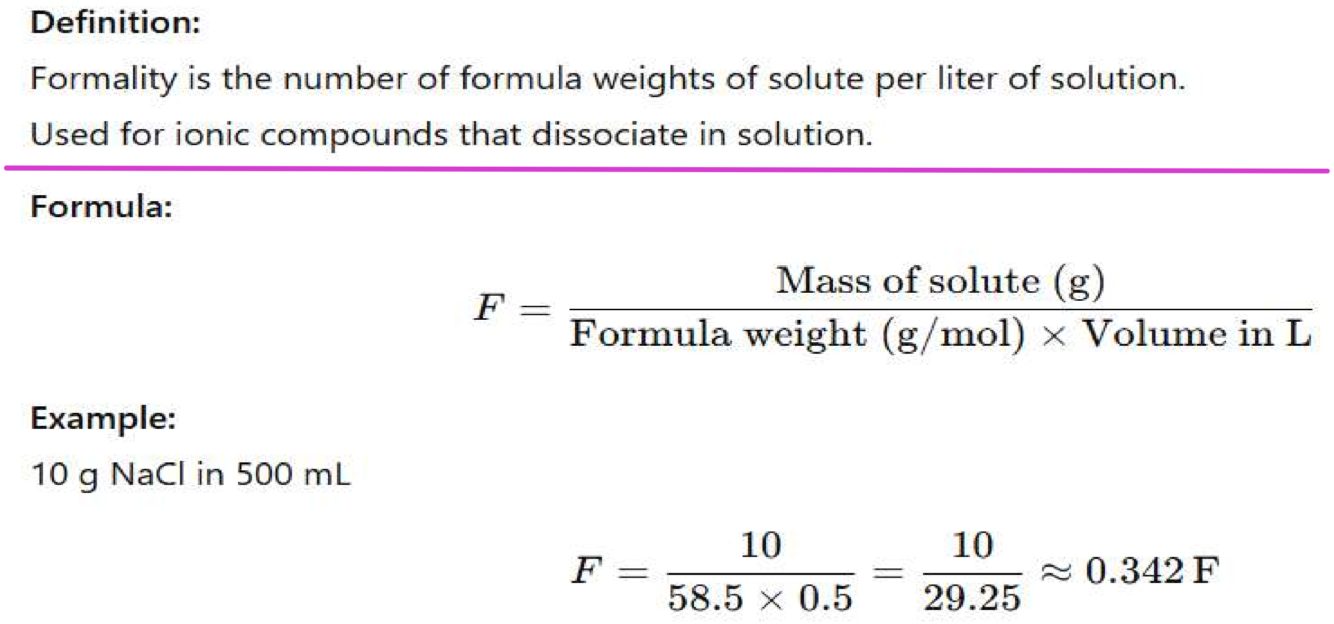
Types and Formulas:

- % **w/w** = (Mass of solute / Mass of solution) × 100
- % **w/v** = (Mass of solute / Volume of solution in mL) × 100
- % **v/v** = (Volume of solute / Volume of solution in mL) × 100

Example:

Prepare 5% w/v NaCl solution = 5 g NaCl in 100 mL solution

5. Formal Concentration (F)



Definition:

Formality is the number of formula weights of solute per liter of solution.

Used for ionic compounds that dissociate in solution.

Formula:

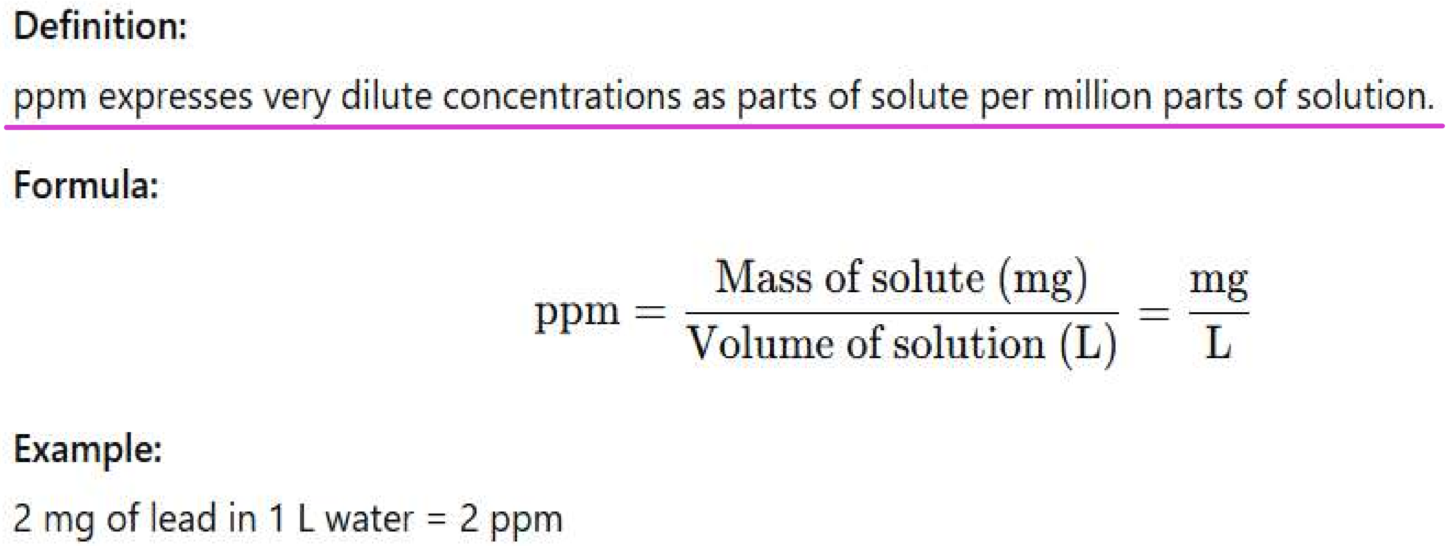
$$F = \frac{\text{Mass of solute (g)}}{\text{Formula weight (g/mol)} \times \text{Volume in L}}$$

Example:

10 g NaCl in 500 mL

$$F = \frac{10}{58.5 \times 0.5} = \frac{10}{29.25} \approx 0.342\,\mathrm{F}$$

6. Parts Per Million (ppm)



Definition:

ppm expresses very dilute concentrations as parts of solute per million parts of solution.

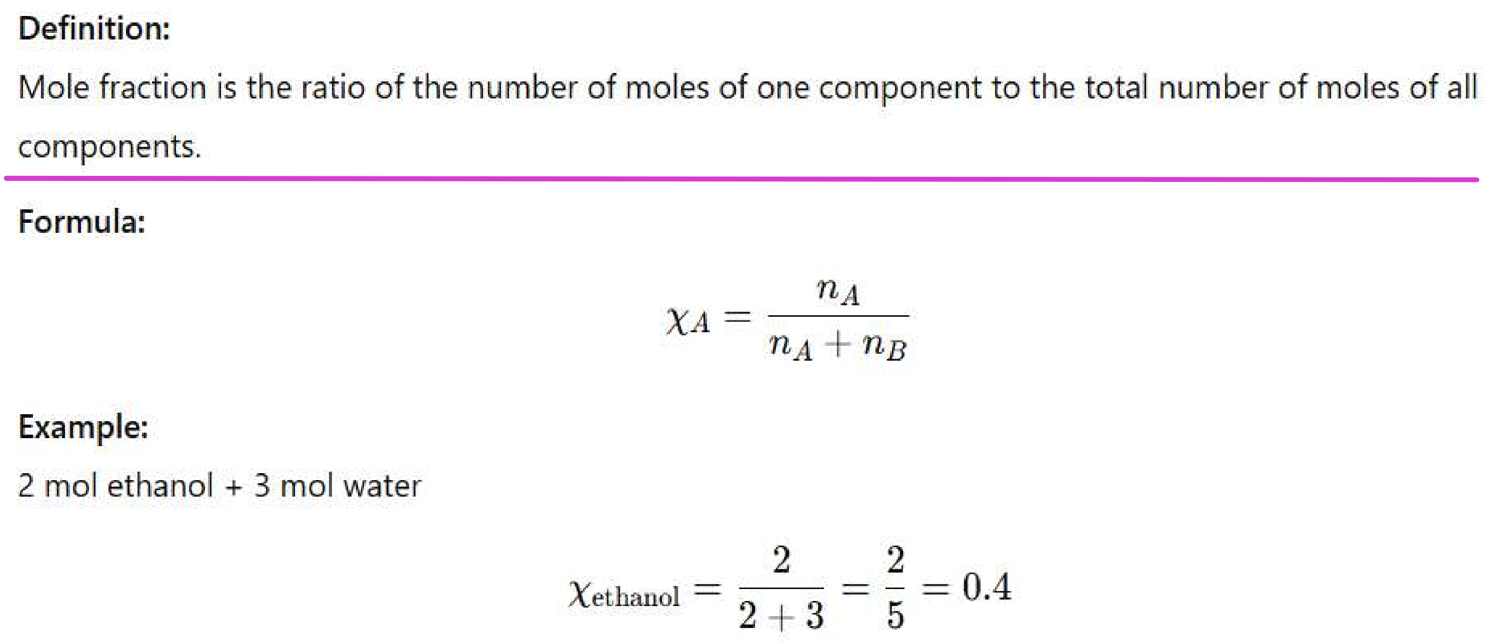
Formula:

$$\mathrm{ppm} = \frac{\text{Mass of solute (mg)}}{\text{Volume of solution (L)}} = \frac{\mathrm{mg}}{\mathrm{L}}$$

Example:

2 mg of lead in 1 L water = 2 ppm

7. Mole Fraction (x)



Definition:

Mole fraction is the ratio of the number of moles of one component to the total number of moles of all components.

Formula:

$$\chi_A = \frac{n_A}{n_A + n_B}$$

Example:

2 mol ethanol + 3 mol water

$$\chi_{\text{ethanol}} = \frac{2}{2+3} = \frac{2}{5} = 0.4$$

Primary and secondary standards

In pharmaceutical analysis, **standards** are substances used to **calibrate analytical methods** or determine the **strength/purity** of analytes. They are classified into **primary** and **secondary standards** based on their **purity**, **stability**, and **use**.

Primary Standard

Definition:-A **primary standard** is a **high-purity**, **stable**, and **non-hygroscopic** substance that can be **weighed directly** to prepare a solution of **known concentration** without prior standardization.

Characteristics:

- ❑ Very **high purity** (≥ 99.9%)
- ❑ **Stable** and not affected by air or moisture
- ❑ **Known chemical formula and molecular weight**
- ❑ **Soluble** in desired solvent
- ❑ Undergoes **complete and known reaction**

Examples:

- ❑ Sodium carbonate (Na_2CO_3) – for acid-base titrations
- ❑ Potassium hydrogen phthalate (KHP)
- ❑ Arsenic trioxide (As_2O_3) – for redox titrations
- ❑ EDTA – for complexometric titrations

Secondary Standard

Definition:-A **secondary standard** is a solution whose concentration **cannot be accurately prepared by weighing** the solute directly and **must be standardized** using a primary standard.

Characteristics:

- ☐ May be **impure** or **hygroscopic**
- ☐ **Unstable** over time
- ☐ Used in **routine analysis**
- ☐ Requires **frequent standardization**

Examples:

- ☐ Hydrochloric acid (HCl)
- ☐ Sodium hydroxide ($NaOH$)
- ☐ Potassium permanganate ($KMnO_4$)
- ☐ Iodine solution (I_2)

Preparation and standardization of various molar and normal solutions

2. Oxalic Acid ($H_2C_2O_4 \cdot 2H_2O$) – *Primary Standard*

✓ **Preparation (0.1 N):**

- **Molecular weight:** 126 g/mol
- **Equivalent weight:** 63 g/eq (donates 2 H^+)
- **Weigh 6.3 g**, dissolve in water, and make up to **1 litre**.

✓ **Standardization:**

- Primary standard – **no need to standardize**.
- Used to standardize **NaOH** and **$KMnO_4$**.

2. Sodium Hydroxide (NaOH) – *Secondary Standard*

✓ **Preparation (0.1 N):**

- **Molecular weight:** 40 g/mol
- Weigh **4.0 g**, dissolve in **CO_2-free distilled water**, and make up to **1 L**.

✓ **Standardization:**

- Use **0.1 N oxalic acid** as primary standard
- **Indicator:** Phenolphthalein (colorless → pink)

Reaction:

$$H_2C_2O_4 + 2NaOH \rightarrow Na_2C_2O_4 + 2H_2O$$

3. Hydrochloric Acid (HCl) – *Secondary Standard*

✓ **Preparation (~0.1 N):**

- Use **8.3 mL** of concentrated HCl (approx. 36%)
- **Dilute with water** and make up to **1 litre** (in fume hood).

✓ **Standardization:**

- Use **0.1 N Na_2CO_3**
- **Indicator:** Methyl orange (yellow → orange)

•**Reaction:**

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

4. Sodium Thiosulphate ($Na_2S_2O_3\cdot 5H_2O$) – *Secondary Standard*

✓ **Preparation (0.1 N):**

- **Molecular weight:** 248 g/mol
- Weigh **24.8 g**, dissolve in water, make up to **1 L**.
- Store in **amber bottle** with 1 g Na_2CO_3 as preservative.

✓ **Standardization:**

- Use **iodine solution** or **potassium dichromate**
- **Indicator:** Starch (blue → colorless)

•**Reaction:**

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

5. Sulphuric Acid (H_2SO_4) – *Secondary Standard*

✓ **Preparation (~0.1 N):**

- Use **1.4 mL** of concentrated H_2SO_4 (98%, d = 1.84 g/mL)
- Add **slowly to water**, make up to **1 L**.

✓ **Standardization:**

- Use **0.1 N Na_2CO_3**
- **Indicator:** Methyl orange
- Same reaction as HCl with Na_2CO_3

6. Potassium Permanganate ($KMnO_4$) – *Secondary Standard*

✓ **Preparation (0.1 N):**

- **Equivalent weight:** 31.6 g/eq
- Weigh **3.16 g**, dissolve in water, heat gently, cool, let stand 24 hrs, and filter.

✓ **Standardization:**

- Use **0.1 N oxalic acid** (heated to 60–70°C)
- **Self-indicator** (pink color persists)

Reaction:

$$2MnO_4^- + 5H_2C_2O_4 + 6H^+ \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$$

7. Ceric Ammonium Sulphate [$(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$] – *Secondary Standard*

✓ **Preparation (0.1 N):**

- **Equivalent weight:** ≈ 548 g/mol
- Weigh **54.8 g**, dissolve in **1% H_2SO_4**, and make up to **1 L**.

✓ **Standardization:**

- Use **ferrous ammonium sulphate (FAS)**
- **Indicator:** Ferroin or Diphenylamine sulfonate

•**Reaction:**

$$Ce^{4+} + Fe^{2+} \rightarrow Ce^{3+} + Fe^{3+}$$

Thank You

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