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GPAT

GRADUATE PHARMACY APTITUDE TEST

NATIONAL TESTING AGENCY (NTA)

VOLUME – I PART - 3

PHARMACUETICAL CHEMISTRY

PHARMACUETICAL ANALYSIS & CHROMATOGRAPHY

SIERRA INNOVATIONS

CONTENT

PHARMACEUTICAL ANALYSIS

PHARMACOGNOSY

BIOTECHNOLOGY

Pharmaceutical Analysis

Definition, Type and scope

A. **Definition**: It is a technique to identify or/and quantify any sample, compound or substance by using manual method, chemical method, Instrumental method.

B. **Type of Pharmaceutical Analysis**:

1. Qualitative Analysis
2. Quantitative Analysis

a. **Qualitative Analysis**: (determine present or absent) completely unknown sample is taken & analysed to presence or absence of the particular substance.

b. **Quantitative Analysis**: Determine of the quantity in number, weight, length or any other measurement parameter. Exact quantity of the sample is quantified is this method.

C. **Classification of Pharmaceutical Analysis Laboratory**:

a. Government Regulatory Agencies, Established by central & state govt. and these are continuously monitoring and analysis the drug sample, e.g: IPC, CDSCO (Central drug standard central organisation)

b. Manufacturer of drugs

c. Manufactures of Row material of drug

d. Universities and Non-commercial Research centers

f. Consulting Laboratories

D. SCOPE:

a. Examination of raw material

b. Analysis of various drugs sample,

c. Qualitative quantitative analysis of sample

d. diagnosis of various disease by chemical Analysis.

e. Determination of Radio active compound.

f. Determination of Natural phytoconstiment.

g. Determination of different sample of water.

Pharmaceutical Analysis

- Instrumental method
  - Volumetric method
  - Spectral Analysis
  - Chromatographic tech.
  - Electro chemical method
  - Hyphenated tech.
  - Other method
- Non-Instrumental method
  - Biological method
    - Invitro method
    - Animal study
    - Microbiological assay
  - Chemical method

    Various chemical test e.g. ch. test for alkaloid, glucose phenal.

Instrumental method

A. Volumetric Method (Titrimetia method)

1. Acid Base- A.q
2. Redox titration/ oxidation - Reduction titration
3. Complexo metric titration
4. Precipitation titration

B. Spectral Analysis

1. Colorimetry (covered the visible range) (Measured by visible light) 400-800nm
2. UV- visible spectroscopy
3. Infra Red (IR) spectroscopy (determination of function group)

4. NMR Spectroscopy (determined the structure of compound, H and C etc.)

5. Mass spectroscopy

6. Spectro fluorometry

C. Chromatographic technique

1. Planner Chromatography → Paper Chromatography

→ TLC

→ HPTLC

2. Column Chromatography

Column (Gravity) Chromatography

Gas Chromatography

Flash Chromatography

HPLC

Size Exclusion Chromatography

Ion Exchange Chromatography

Affinity Chromatography

UPLC (Utra performance liquid Chromatography)

DCCC (Droplet counter current Chromatography)

D. Electro chemical Method

1. Conductometry

2. Coulometry

3. Voltametry

4. Potentiometry

E. Hyphenated technique

1. GC - MS (Gas Chromatography - mass spectroscopy)

2. LC - MS
3. LC - NMR
4. GC - NMR
5. LC - MS - MS
6. ICP - OES (inductive couple of plasma - optical emission spectroscopy)
7. ICP - AAS
8. ICP - MS

F. Other Method
1. DSC (differential scanning calorimetry)
2. TGA (measured Thermal properties) (Thermo gravimetry Analysis)
3. Kjeldehl method (N2 content in tho organic compound)
4. RIA (Radio Emission Assay determine Antigen present in the serum of the patient
3. Auto radiography
4. Flame photometry (determine many Elements) → like C, H, N, O, S
5. Elemental Analysis

Normality (N)

No. of gram equivalent of solute present per liter of sol^n (Detonated by 'N')

$$\boxed{N = \frac{\text{No. of gram equivalent of sulute}}{\text{vol of sol}^n \text{ (in ltr)}}} = gm/L$$

= gram equivalent

A. Gram equivalent of Acid = $\frac{M.W}{\text{No. of replacement ion or Basicity of the acid}}$

e.g. HCl → H^+ + Cl^- (replacement)

$H_2SO_4 \longrightarrow 2H^+ + SO_4^{2-}$ (Two replacement ion)

Gram eq. = $\frac{M.w}{\text{No.of replacement}} = \frac{98}{2}$ = 1N

In case of dibasic acid 1N = 1/2 M

In case of tribasic acid 1N = 1/3 M

B. Gram equivalent of Acid = $\frac{M.W}{\text{No. of replacement ion or Basicity of the acid}}$

$$NaOH \longrightarrow Na^{+} + OH^{-} = \frac{40}{1} = 40g/L = 1N$$

$$Na_2Co_3 \rightarrow 2N_a^{+} + Co_3^{2-} = \frac{106}{2} = 53g/L = 1N$$

C. Gram equivalent in Redox Rx^n $\frac{\text{M.W}}{\text{Change in the Oxidation number}}$

$$KM_nO_4 \rightleftharpoons K^{+} + M_nO_4^{-}$$

In Acidic medium $\underset{(+7)}{M_n}\underset{(-8)}{O_4^-} + \underset{(1)}{e} \rightarrow \underset{(+6)}{M_n}\underset{(-8)}{O_4^{2-}}$

eq wt $\frac{158}{1}$ = 158g/L = 1 N

Neutial Medium $\underset{(+7)}{M_n}O_4^- + 4H^+ + \underset{(3)}{3e} \rightarrow \underset{+4(-4)}{M_nO_2} + 2H_2O$

eq wt $\frac{158}{3}$ = 52.66g/L = 1 N

D. Acidic Medium $\underset{(17)}{M_n}O4 + 3H^+ + \underset{(5)}{5e^-} \rightarrow \underset{(12)}{M_n^{2+}} + 4H_2O$

eq wt $\frac{158}{3}$ = 31.69g/L = 1 N

Molarity and Molality

Molarity: Number of mole of the solute present is one litre of the sol^n.

Denoted by 'M'.

$$M = \frac{\text{No.of Moles of solute}}{\text{Vol.of sol}^n\text{(in litre)}} = \text{mole/L}$$

Volume may changed due to temp. so it is temp. dependent.

$$\text{No. of mole} = \frac{\text{Mass given}}{\text{Gram atomic mass}}$$

E.g.(1) What is the molarity of sol^n prepared by dissolving 15 g of NAOH in enough water to make total of 225 ml of sol^n.

$$\text{Molarity} = \frac{\text{No. of mole of solute}}{\text{Vol. of sol}^n \text{ (in ltr)}}$$

$$\text{No. of mole of solute} = \frac{\text{Mass given of NaOH}}{\text{Mass is given of NaOH}}$$

$$= \frac{15}{40} = 0.375 \text{ mole NaOH}$$

$$\text{Molarity of NaOH} = \frac{0.375\,mole}{0.225\,l}$$

$$= 1.67 \text{ M NaOH}$$

Prep^n of 1 M NaOH sol^n = 40g of NaOH will be dissolved in the sufficient amount of the distilled water and final value will be 1000 ml.

E.g(2) What is the molarity of a sol^n that contain 1.724 mole of H_2SO_4 in 2.50L of sol^n.

$$\text{Molarity} = \frac{\text{No. of mole of solute}}{\text{Vol. of sol}^n \text{ (in ltr)}}$$

$$= \frac{1.724\ mole}{2.50}$$

$$= 0.688 \text{ M of } H_2SO_4$$

E.g(3) What is the molarity of sol^n prepared by dissolving 250 g of HCL (g) in enough water to make 150.0 ml of sol^n.

$$\text{Molarity} = \frac{\text{No. of mole of solute}}{\text{Vol. of sol}^n \text{ (in ltr)}}$$

$$\text{Mole} = \frac{\text{Mass given}}{\text{Gram atomic mass}}$$

$$= \frac{25}{36.5}$$

Mole = 0.684 Mole of HCL

$$= \frac{0.684\ mole}{0.150\ l}$$

= 4.56 M of HCL

E.g (4) How many g of NaOH present in 400 ml of 0.5M sol^n of NaOH.

$$\text{Molarity} = \frac{\text{No. of mole of solute}}{\text{Vol. of sol}^n\ \text{(in ltr)}}$$

1000 ml of 1 M NaOH = 40g

500 ml of 0.5M NaOH = 20g

400 ml of 0.5M NaOH = 0.5m

$$= 2\not{0} \times \frac{4\not{0}\not{0}}{1\not{0}\not{0}\not{0}}$$

= 8 gm

Molality (m)

No. of moles of the solute present in 1 kg of the solvent.

It is rarely used term $= \frac{\text{mass}}{\text{wt}}$

$$\text{Molarity} = \frac{\text{No. of mole of solute}}{\text{Mass of the solvent (in kg)}}$$

Molality is temp. independent because mass/wt of the solvent will not change due to temp.

$Prep^n$ of 1 m NaOH Sol^n 40g of NaOH will be dissolved in the 1 kg of the distilled water. So the final volume may be more than 1 kg.

Formal Concentration (f)

- It is calculated based on the formula wt of the chemicals per ltr of the sol^n
- Denoted by 'f'
- It is rarely used term.

$$F = \frac{\text{Formula wt of the solute}}{\text{Vol. of the sol}^n \text{ (in ltr)}}$$

Formal Con^c indicate moles of the original chemical formula in the soln without considering the species actually exist in the sol^n.

Molar Con^c is the conc^n of the species which is actually exist in the sol^n.

Example (1) $Na_2CO_3 \rightarrow 106g \rightarrow 1M$

$Na_2CO_3 \rightarrow 2Na^+ + Co_3^{2-}$ (in distilled water)

In water some amount of CO^{2-}_3 will convert in to HCO^-_3 and H_2CO_3

Acidic medium $Co_3^{2-} + H^+ \rightarrow HCo_3^-$

$HCo_3^- + H^+ \rightarrow H_2Co_3$

Example (2) $CaCO_3 \rightarrow 100g$

$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-}$ (in distilled water)

In water some amount of CO^{2-}_3 will convert in to HCO^-_3 and H_2CO_3

Acidic medium $CO_3^{2-} + H^+ \rightarrow HCO_3^-$

$HCO_3^- + H^+ \rightarrow H_2CO_3$

Percent Concentration (%w/w, %v/v, %w/v)

Many time conc^n is expressed in term of **%** or part per hundred.

A. $\% w/w = \frac{\text{Wt of the solute ing}}{\text{Wt of the sol}^n \text{ ing}} \times 100$

Example (1) preparation of 10% w/w NaCl.

$$\% w/w = \frac{10 \text{ g of NaCL}}{100 \text{ g the sol}^{n}} \times 100$$

% w/w = 10% NaCL

B. $\% v/v = \frac{\text{Vol. of solute (in ml)}}{\text{Vol. of sol}^{n} \text{ (in ml)}} \times 100$

Example (2) preparation of 10% v/v acetic acid.

$$\% v/v = \frac{10 \text{ ml acetic acid}}{100 \text{ ml sol}^{n}} \times 100$$

% v/v of acetic acid. = 10%

C. $\% w/v = \frac{\text{mass of the solute}}{\text{Vol. of the sol}^{n} \text{(in ml)}} \times 100$

Example (3) preparation of 10% w/v NaOH sol^n

$$\% w/v = \frac{10\text{g NaOH}}{100 \text{ ml sol}^{n}} \times 100$$

= 10 % w/v (g/m)

Example (4) Calculate the normality of the 20% w/v NaOH sol^n.

20g NaOH → 100 ml of sol^n

4g of NaOH in 100 ml = 1 N NaOH

20g of NaOH in 100 ml

$$= \frac{20}{4} = 5N \text{ NaOH}$$

Example (6) 10 g of NaCl is present in 100 mg of sol^n. find wt/w%. If density of sol^n is 1.2g /m/

$$\text{wt/wt\%} = \frac{\text{mass of solute}}{\text{mass of sol}^{n}} \times 100$$

$$= \frac{10 \text{ g}}{120 \text{ g}} \times 100 = 8.33\%$$

d(f) = m/v ⇒ 1.2 = m /100 ⇒ 1.2 × 100 = m

m = 120 g

Example (7) A sugar sol^n is 10% (w/v). Find (w/w%) if d of sol^n = 125g

10 g sugar in 100 ml

$$f = \frac{m}{v} \Rightarrow 1.25 \times \frac{m}{100} = 125g$$

$$\text{w/w\%} = \frac{\text{mass of solute}}{\text{mass of sol}^{\text{n}}} = \frac{10}{125} \times 100 \Rightarrow 8\% w/w$$

PPM (Parts per million) & PPB (Parts per Billion)

PPM: It is frequently used to express the conc^n of very solute sol^n.

It is also used to express the conc^n of impurities in the pharmaceuticals.

$$\text{ppm} = \frac{\text{Mass of solute (ing)}}{\text{Mass of sol}^n\text{ (ing)}} \times 10^6$$

Example (1) Prep^n of 100 PPM of chloride from NaCl.

$$\text{Chloride 100 PPM } \frac{100}{1000000} \times 100\%$$

Chloride 100 PPM = 0.01%

M.W. of NaCl = 56.5, Atomic wt of Cl = 35.5

$$\text{Cl 100 PPM } = \frac{56.5}{35.5} \times 0.01$$

= 0.01648 g dissolved in 100 ml of distilled water

Example (2) Conc^n of Na_2CO_3 in sea water is 53 PPM find mass of Na_2CO_3 present in 250 ml of water.

$$\text{PPM } = \frac{\text{mass of solute in gram}}{\text{mass of sol}^{\text{n}} \text{ in gram}} \times 10^6$$

PPM = 53

Mass of solute in gram = ?

Mass of sol^n in gram = 250 g

$$53 = \frac{\overset{Na_2CO_3}{\text{mass of solute in gram}}}{250} \times 10^6$$

53 × 250

13250×10^{-6} gram = mass of Na_2CO_3 in 250g of water.

$1.325 \times 10^{-2} = 0.01325$

PPB → Parts per Billion = 10^9

$$\boxed{\text{no. of } PPB = \frac{\text{Mass of solute}}{\text{mass of solution}} \times 10^9}$$

Relation between Normality and molarity

$X = \frac{N}{M}$ X = No. of OH^- or H^+ ion

N = Normality

M = Molarity

$$\boxed{N = M \times X}$$

$$M = \frac{N}{X}$$

Ex. Find normality of 0.4M H_3PO_4 Sol^n

N = MX

N = ?

M = 0.4

N = 0.4 × 3 = 12 N

Mole fraction (X) (η)

no. of moles $\frac{1}{4}$ $\frac{3}{4}$

Q. A Sol^n has 46 **%** (w/w) ethanol in water. Find mole fraction of ethyl alcohol.

$$\text{Mole fraction of } C_2H_5OH = \frac{\text{No. of mole of } C_2H_5OH}{\text{No. of mole of } C_2H_5OH + \text{No. of mole of } H_2O}$$

$$\text{No. of mole of } C_2H_5OH = \frac{\text{mass given}}{\text{Molecular mass}} = \frac{46}{46} = 1$$

$$\text{No. of mole of } H_2O = \frac{100-46}{18} = \frac{54}{18} = 3$$

$$\text{Mole fraction of } C_2H_5OH = \frac{1}{1+3} = \frac{1}{4} = 0.25$$

$$\text{Mole fraction of } H_2O = \frac{3}{1+3} = \frac{3}{4} = 0.75$$

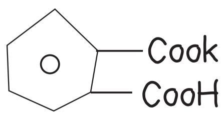
Primary Standard Substance

Primary Standard substance

- Highly pure reagent/chemical are used to prepare standard sol^n, which doesn't requires further standardization is known as primary standard sol^n.
- It should be purified dried & easily available. They must stable & free from hygroscopic nature.
- 100% purity (if impurities present them 0.01-0.02%)
- High molecular weight to reduce the weighing errors.

NaOH → 40 → 0.1N → 4g /L → 4.1g, 3.9 g ± 0.1 g

$$= \frac{0.1}{4} \times 100 = 2.5\% \text{ error is high}$$

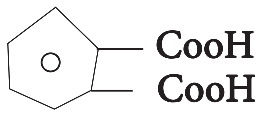
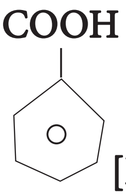
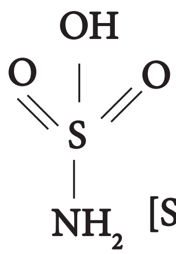


→ 204 → 0.1 → 20.4 ± 1 g

$$= \frac{0.1}{20.4} \times 100 = 0.5 \text{ error is low}$$

- It should be completely dissolved under experimental condition.
- Free from any hydrated water moiety
- Primary standard substance should not be able to satisfy all the above mentioned characteristic so closely relevant substance are considered as primary standard substances.

Primary standard substance for Acid Base Titration →

Compound Name	M.w
1. Na_2CO_3	106
2. COOH – COOH $2H_2O$ (Oxalic acid)	126
3.  [phthalic acid]	204
4. CH_2COOH – CH_2COOH [Succinic acid]	118
5.  [Benzoic acid]	122
6. $CH_2–CH_2COOH$ – $CH_2–CH_2COOH$ [Adipic acid]	146
7.  [Sulphamic acid]	97
8. $Na_2B_4O_7 - 10H_2O$ 381 [Sod.tetrabrote]	381
9. Constant boiling HCl	36.5

Primary standard Sol^n for Redox Titration →

1. $k_2Cr_2O_7$ Pot.clichromate — 294
2. $KBrO_3$ Pot.Bromate — 167
3. KIO_3 Pot.iodate — 214
4. $Na_2C_2O_4$ Sod.Oxalate — 134
5. As_2O_3 Arsenic.Oxalate — 198
6. $C_4SO_45H_2O$ Copper sulphate (Blue vitrol) — 250

Precipitation titration

1. Ag — 108
2. $AgNO_3$ — 170
3. NaCl — 58.5
4. KCl — 75
5. KBr — 119

Complaxometric Titration

1. Zn — 65
2. mg — 24
3. $ZnCl_2$ — 136
4. $CaCl_2$ — 111

Secondary standard Substances

- A secondary standard is less stable compound of generally used for quantitative analysis of conc^n of sec standard is determined by comparing with primary standard sol^n

- Secondary standard sol^n is titrated with primary standard Sol^n to determine the $conc^n$ of sec stand. sol^n
- Standardization of sec. Stand sol^n is must before doing any quantitative analysis of pharmaceuticals
- Standardized sec. sol^n if used. to determine the $conc^n$ of analyzer.

Acid Base Titration

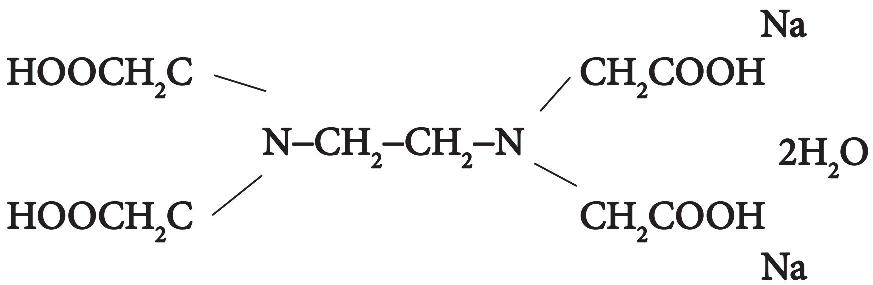
1. HCl → 36.5
2. H_2SO_4 → 98
3. NaOH → 40
4. $HClO_4$ → 100

Redox Titration

1. KM_nO_4 15.8
2. $(NH_4)_3$ $Ce(SO_4)_2$ $2H_2O$ 633
3. $Na_2S_2O_3$ 158

Complaxometric Titration

1. EDTA → 372 → (Sec std. Substance)



2. Pb $(NO_3)_2$ 331