Advanced Pharmaceutical Analysis
(UNIT - I)

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DIAZOTIZATION METHODS

Diazotization methods

- The process of forming diazonium compounds or salts is called diazotation, diazoniation, or diazotization.
- Diazonium compounds or diazonium salts are a group of organic compounds sharing a common functional group with the characteristic structure of R-N2 + X- where R can be any organic residue such alkyl or aryl and X is an inorganic or organic anion such as a halogen.
- Diazotization titrations are carried out for the estimation of drugs containing primary aromatic amino group.
- Several drugs contain either primary aromatic amino group or they can be converted to have such groups by simple reaction like hydrolysis or reduction.

- An primary aromatic amine reacts with nitrous acid produced by the reaction of sodium nitrite in acidic medium to form diazonium salt.
- The reaction is quantitative under the controlled conditions of temp. (approx 150C) and the end point can be detected when a small quantity of excess nitrous acid present at the end point gives colour change with indicator or by electromerically.
- It uses the titrant- Sodium Nitrite hence method is Soduim Nitrite Titration / Nitrite Titration

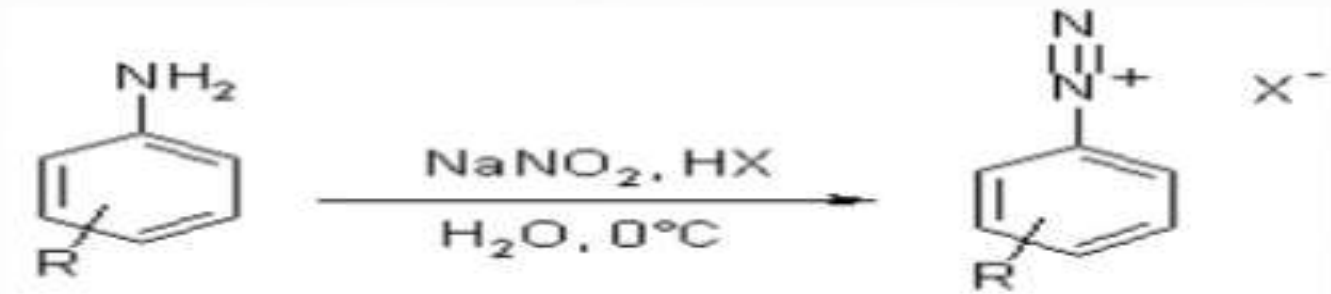
Principle

- The first involved is addition of sodium nitrite to hydrochloric acid cause formation of nitrous acid

$$NaNO_2 + HCl \longrightarrow HONO + NaCl$$

- This nitrous acid diazotises the aromatic amino group

$$R - NH_2 + NaNO_2 + HCl \longrightarrow R - N^+ \equiv N - Cl^- + NaCl + ..$$



- After the end point , excess nitrous acid formed is shown by instant formation of blue colour with starch iodide paper.

$KI + HCl \longrightarrow HI + KCl$

$NaNO_2 + HCl \longrightarrow HNO_2 + NaCl$

$2HI + 2HONO \longrightarrow I_2\uparrow + 2NO + 2H_2O$

- Starch iodide paper is prepared by immersing a filter paper in starch mucilage and potassium iodide solution

- The iodine formed reacts with starch mucilage to give the blue colour.

$I_2 + \text{Starch} \longrightarrow \text{blue color (end point)}$

Procedure

- Weigh accurately 0.5 g sulphonamide add to it 20 ml of hydrochloric acid and 50 ml water, stir, dissolve and cool to 150c. Immerse the electrode in the solution and apply the voltage of about 50 mV.
- Place burette tip just below the solution to eliminate oxidation of sodium nitrite. Stir it gently & maintain the temp below 150c.

NON AQUEOUS TITRATION

NON-AQUEOUS TITRATION

- Nonaqueous titration is the titration of substances dissolved in solvents other than water.
- It is the most common titrimetric procedure used in pharmacopoeia assays and serves a double purpose: it is suitable for the titration of very weak acids and very weak bases, and it provides a solvent in which organic compounds are soluble.
- The most commonly used procedure is the titration of organic bases with perchloric acid in anhydrous acetic acid.

ASSAY BY NON-AQUEOUS TITRATIONS

- Acidimetry in Non-aqueous Titrations—It can be further sub-divided into two heads, namely :

(i) Titration of primary, secondary and tertiary amines, and

(ii) Titration of halogen acid salts of bases.

- Alkalimetry in Non-aqueous Titrations— titration of acidic substances

ACIDIMETRY IN NON AQUEOUS TITRATIONS

Example : Primary amines

METHODOLOGY: four steps

- (i) Preparation of 0.1 N Perchloric acid,
- (ii) Standardization of 0.1 N Perchloric Acid,
- (iii) Choice of Indicators

PREPARATION OF 0.1 N PERCHLORIC ACID

- Gradually mix 8.5 ml of perchloric acid to 900 ml of glacial acetic acid with vigorous and continuous stirring. Now add 30 ml acetic anhydride and make up the volume to 1 litre with glacial acetic acid and allow to stand for 24 hours before use.
- The acetic anhydride reacts with the water (approx. 30%) in perchloric acid and some traces in glacial acetic acid thereby making the resulting mixture practically anhydrous. Thus, we have :

$$H_2O + (CH_3CO)_2O \rightarrow 2CH_3COOH$$

Acetic anhydride Acetic acid

STANDARDIZATION OF 0.1 N PERCHLORIC ACID

Weigh accurately about 0.5 g of potassium hydrogen phthalate in a 100 ml conical flask. Add 25 ml of glacial acetic acid and attach a reflux condenser fitted with a silica-gel drying tube. Warm until the salt gets dissolved completely. Cool and titrate with 0.1 N perchloric acid by making use of either of the following two indicators :

(a) acetous crystal violet-2 drops, end point Blue to Blue- Green (0.5% w/v)

(b) acetous oracet blue B-2 drops, end point Blue to Pink.

Titration of primary amines

Ex: Methlyldopa

$R.NH_2 + HClO_4 \rightarrow [R.NH_3]^+ + ClO_4^-$

Procedure : Weigh accurately about 0.2 g and dissolve in 15 ml of anhydrous formic acid, 30 ml of glacial acetic acid and 30 ml of dioxane. Add 0.1 ml of crystal violet solution and titrate with 0.1 N perchloric acid. Perform a blank determination and make any necessary correction.

Calculations

0.02112 g $C_{10}H_{13}NO_4 \equiv$ 1 ml of 0.1 N $HClO_4$

The percentage of methyldopa present in the sample is given by :

$$\% \text{ Methyldopa} = \frac{ml \times 0.1 \times 0.02112 \times 100}{\text{wt. of sample}}$$

Assay of metronidazole

- **PRINCIPLE:** Metronidazole is an example of anti-amoebic, anti-trichomonal and anti-giardial drug. It is widely used in the management of amoebiasis, thrichomoniasis and giardiasis. It is estimated by non-aqueous titration. In this method, solution of metronidazole in glacial acetic acid is titrated against standard solution of acetous perchloric acid (0.1N) using crystal violet as indicator.
- **PROCEDURE:**
- **Procedure for Standardization:** Standardize the 0.1N perchloric acid using standard solution of potassium hydrogen phthalate (0.1N) and crystal violet indicator.
- **Procedure for Assay:** Weigh accurately about 0.45g of metronidazole and add dissolve in 10ml of glacial acetic acid. Add few drops of crystal violet solution and titrate with 0.1N perchloric acid until a pale green color is produced. Perform a blank determination and make any necessary correction.
- Each ml of 0.1N perchloric acid is equivalent to 0.01712g of metronidazole.
- From the volume of 0.1N perchloric acid consumed, calculate the amount of metronidazole present in the given sample.

Assay of salbutamol

- **PRINCIPLE:** Salbutamol is an example of anti-asthmatic drug. It is estimated by non-aqueous titration. In this method, solution of salbutamol in glacial acetic acid is titrated against standard solution of acetous perchloric acid (0.1N) using oracet blue B as indicator.
- **PROCEDURE:**
- **Procedure for Standardization:** Standardize the 0.1N perchloric acid using standard solution of potassium hydrogen phthalate (0.1N) and crystal violet indicator.
- **Procedure for Assay:** Weigh accurately about 0.9g of salbutamol sulphate and add dissolve in 10ml of glacial acetic acid. Add few drops of crystal violet and titrate with 0.1N perchloric acid.
- Each ml of 0.1N perchloric acid is equivalent to 0.05767g of salbutamol sulphate.

Other examples for non-aqueous titrations

S.No.	Name of the substance	Indicator employed
1	Amantadine hydrochloride	Crystal violet
2	Chlorpromazine hydrochloride	Methyl orange
3	Clonidine hydrochloride	A – naphthol benzein
4	Cyproheptadiene HCl	Crystal violet
5	Ephedrine HCl	Crystal violet
6	Nalidixic acid	Thymolphthalein
7	Mercaptopurine	Thymol blue

REDUCTION-OXIDATION METHODS

Assay of ascorbic acid

- **Principle:** Ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colorless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink colour in acidic medium. Oxalic acid is used as the titrating medium.
- **Procedure:**

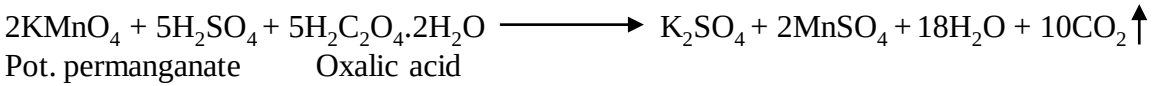
1. Pipette out 5ml of the working standard solution into a 100ml of conical flask.
2. Add 10ml of 4% oxalic acid and titrate against the dye (V1 ml). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge.
4. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V2 ml).

Assay of Isoniazid

- **PRINCIPLE:** Isoniazid is an example of antitubercular drug. It is estimated by redox titration (iodometry). Isoniazid is treated with excess of 0.1N bromine solution. The excess of bromine not required for oxidation is determined by adding potassium iodide and titrating the liberated iodine with standard solution of 0.1N sodium thiosulphate and starch indicator.
- **PROCEDURE:**
- **Procedure for Standardization:** Standardize the sodium thiosulphate 0.1N using standard solution of potassium iodate (0.1N) and starch indicator. Later standardize the 0.1N bromine solution using the standardized sodium thiosulphate solution.
- **Procedure for Assay:** Weigh accurately about 0.4g of isoniazid and add dissolve in water to produce 250ml. Transfer 25ml of this to an iodine flask, add 25ml of 0.1N bromine solution and cool to about 15ºC. Add 5ml of hydrochloric acid, shake for 1 minute and allow to stand for 15 minutes in water bath maintained at about 15 ºC. Add 10ml of potassium iodide solution and titrate with 0.1N sodium thiosulphate solution using starch solution as indicator. Carry out blank determination.
- Each ml of 0.1N bromine is equivalent to 0.003429g of Isoniazid.

Assay of hydrogen peroxide

- **PRINCIPLE:** The assay of hydrogen peroxide is based on redox titrations. Potassium permanganate is a strong oxidizing agent and oxalic acid is a reducing agent. The reaction between potassium permanganate and oxalic acid tends to proceed slowly. Hence warming at 70ºC is required.



- Hydrogen peroxide and acidified potassium permanganate both are oxidizing agents. These two oxidizing agents reduce one another with the evolution of oxygen gas. Hydrogen peroxide reduces potassium permanganate and causes its decolorization. At the end point, the excess drops of potassium permanganate give pink color. Potassium permanganate itself acts as a self indicator.

PROCEDURE:

- **Preparation of 0.02N potassium permanganate solution:** 0.63g of potassium permanganate is dissolved in 1lt of water. Heat it on water bath for 1hr and allow standing for 2 days and filter the solution.
- **Preparation of 0.02N oxalic acid solution:** Dissolve 1.26g of oxalic acid in 1000ml of distilled water.
- **Preparation of 1M sulphuric acid solution:** Dilute 54ml of sulphuric acid in 1000ml of distilled water.
- **Standardization of 0.02N potassium permanganate solution:** Pipette out 10ml of 0.02N oxalic acid solution into a clean conical flask and add 10ml of dilute sulphuric acid and boil the contents of the flask upto 70ºC. Titrate the flask against 0.02N potassium permanganate until a fair pink color obtained and persists about 30 seconds.
- **Assay of hydrogen peroxide:** To 1.0 ml of hydrogen peroxide, add 20 ml of 1 M sulphuric acid and titrate with 0.02 M potassium permanganate.
- Each ml of 0.02N potassium permanganate is equivalent to 0.001701g of H_2O_2.

COMPLEXOMETRIC METHODS

Assay of magnesium carbonate

- **PRINCIPLE:** Complexometric titration is based on the formation of a complex between analyte and the titrant. The chelating agent EDTA is very commonly used to titrate the metal ions in solution. Thus, titration involves specialized indicator that form weaker complex with the analyte.

In the assay of magnesium carbonate, metal ion indicator mordant black – II is used. It forms a complex with metal ion and with disodium EDTA. The metal – indicator complex should be less stable so that disodium EDTA will take up the metal ion from the metal – indicator complex. This leads to liberation of free indicator to show sharp, rapid color change, so the color of solution changes to blue.

PROCEDURE:

- **Preparation of 0.05M disodium EDTA solution:** Dissolve 18.6g of disodium EDTA in 1000ml of distilled water.
- **Preparation of 0.05M calcium chloride solution:** 5.5g of calcium chloride is dissolved in 1000ml of distilled water.
- **Preparation of pH 10 buffer solution:** Dissolve 70g of ammonium chloride in 570ml of ammonia and make up the volume to 1000ml with distilled water.
- **Standardization of 0.05M disodium EDTA solution:** Pipette out 10ml of 0.05M calcium chloride solution into a clean conical flask and add 5ml of pH 10 buffer solution and add 2 – 3 drops of solochrome black T indicator. Titrate against 0.05M disodium EDTA.
- **Assay of magnesium carbonate:** Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of water and 2 ml of 2 M hydrochloric acid and add 50 ml of water, 10 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium EDTA, using 0.1 g of mordant black II mixture as indicator, until a blue color is obtained.
- Each ml of 0.05M disodium EDTA is equivalent to 0.002015g of $MgCO_3$

Assay of zinc chloride

- **PRINCIPLE:** Complexometric titration is based on the formation of a complex between analyte and the titrant. The chelating agent EDTA is very commonly used to titrate the metal ions in solution. Thus, titration involves specialized indicator that form weaker complex with the analyte.

In the assay of zinc chloride, metal ion indicator eriochrome black T is used. It forms a complex with metal ion and with disodium EDTA. The metal – indicator complex should be less stable so that disodium EDTA will take up the metal ion from the metal – indicator complex. This leads to liberation of free indicator to show sharp, rapid color change, so the color of solution changes to blue.

PROCEDURE:

- **Preparation of 0.1M disodium EDTA solution:** Dissolve 37.2g of disodium EDTA in 1000ml of distilled water.
- **Preparation of 0.1M calcium chloride solution:** 11.1g of calcium chloride is dissolved in 1000ml of distilled water.
- **Preparation of pH 10 buffer solution:** Dissolve 70g of ammonium chloride in 570ml of ammonia and make up the volume to 1000ml with distilled water.
- **Standardization of 0.1M disodium EDTA solution:** Pipette out 10ml of 0.1M calcium chloride solution into a clean conical flask and add 5ml of pH 10 buffer solution and add 2 – 3 drops of solochrome black T indicator. Titrate against 0.1M disodium EDTA.
- **Assay of zinc chloride:** Weigh accurately about 3.0 g, dissolve in 125 ml of water, add 3 g of ammonium chloride and add sufficient water to produce 250.0 ml. To 25.0 ml of the resulting solution add 100 ml of water and 10 ml of pH 10 buffer solution. Titrate with 0.1M disodium EDTA, using eriochrome black T solution as indicator until a deep blue color is obtained.
- 1 ml of 0.1M disodium EDTA is equivalent to 0.01363 g of $ZnCl_2$.

ACID – BASE TITRATION METHODS (NEUTRALIZATION)

Assay of aspirin

PRINCIPLE: Aspirin or acetyl salicylic acid is an example of analgesic and antipyretic, which is widely used in the management of pain. It is estimated by acid – base titration i.e., by acidimetry and alkalimetry. Its determination depends upon the alkaline hydrolysis of aspirin to acetic acid and salicylic acid (sodium salts are formed immediately), followed by back titration of excess alkali using phenol red as an indicator. A blank determination is needed in this assay.

PROCEDURE:

- **Procedure for Standardization:** Standardize the sodium hydroxide using standard solution of oxalic acid (0.5N) and phenolphthalein indicator. Later standardize the hydrochloric acid using the standardized sodium hydroxide solution and indicator is phenolphthalein.
- **Procedure for Assay:** Weigh accurately about 1.5g of acetyl salicylic acid or aspirin and add 50ml of 0.5N sodium hydroxide solution and boil gently for ten minutes. Titrate the excess alkali with 0.5N hydrochloric acid using phenol red as an indicator.
- Repeat the experiment with the same quantities of same reagents in the same manner omitting the acetyl salicylic acid.
- Each ml of 0.5N sodium hydroxide is equivalent to 0.04504g of $C_9H_8O_4$.

Assay of Ibuprofen

- **PRINCIPLE:** Ibuprofen is an example of non-steroidal anti-inflammatory drug (NSAID). It is also having analgesic and antipyretic activity. It is widely used in management of pain and fever. Ibuprofen is aryl acetic acid derivative and is weakly acidic in nature. It is estimated by acid – base titration i.e., by alkalimetry. In this method, the alcoholic solution of ibuprofen is titrated against standard solution of sodium hydroxide (0.1N) using phenolphthalein as an indicator.
- **PROCEDURE:**
- **Procedure for Standardization:** Standardize the sodium hydroxide using standard solution of oxalic acid (0.1N) and phenolphthalein indicator.
- **Procedure for Assay:** Weigh accurately about 0.5g of ibuprofen and dissolve in 100ml of alcohol and titrate with 0.1N sodium hydroxide solution using phenolphthalein as an indicator.
- Each ml of 0.1N sodium hydroxide is equivalent to 0.02063g of $C_{13}H_{18}O_2$.

Assay of Phenytoin

- **PRINCIPLE:** Phenytoin is an example of anti-epileptic drug. It is estimated by acid base back titration. In this method, to the solution of phenytoin, excess amount of sodium hydroxide is added. The unreacted sodium hydroxide is titrated against standard solution of hydrochloric acid (0.1M) using phenolphthalein as indicator.
- **PROCEDURE:**

Procedure for Standardization: Standardize the 0.1M sodium hydroxide using standard solution of oxalic acid (0.1M) and phenolphthalein indicator. Later, standardize 0.1M hydrochloric acid using standardized sodium hydroxide and phenolphthalein indicator.

Procedure for Assay: Weigh accurately about 0.5g of phenytoin and add 40ml of sodium hydroxide. Add few drops of phenolphthalein solution. Titrate with 0.1M hydrochloric acid. Perform a blank determination and make any necessary correction.

Each ml of 0.1M sodium hydroxide is equivalent to 0.02520g of phenytoin.

Assay of Glibenclamide

PRINCIPLE: Glibenclamide is an example of oral anti-diabetic drug. Chemically, it is sulphonyl urea. It is used in the treatment of type 2 diabetis mellitus. The drug is acidic in nature and can be estimated by alkalimetry. Alcoholic solution of glibenclamide is titrated against standard solution of sodium hydroxide (0.1N) using phenolphthalein as indicator.

PROCEDURE:

- **Procedure for Standardization:** Standardize the 0.1N sodium hydroxide using standard solution of oxalic acid (0.1N) and phenolphthalein indicator.
- **Procedure for Assay:** Weigh accurately about 0.5g of glibenclamide and add dissolve in 100ml of hot alcohol. Titrate with 0.1N sodium hydroxide solution using phenolphthalein as indicator.
- Each ml of 0.1N sodium hydroxide is equivalent to 0.0494g of glibenclamide.

Assay of Furosemide

- **PRINCIPLE:** Furosemide is an example of diuretic. It is widely used in the management of hypertension, oedema, and congestive heart failure. It is estimated by alkalimetry. In this method, solution of furosemide in dimethyl formamide is titrated against standard solution of sodium hydroxide (0.1N) using bromothymol blue as indicator.
- **PROCEDURE:**

Procedure for Standardization: Standardize the 0.1N sodium hydroxide using standard solution of oxalic acid (0.1N) and phenolphthalein indicator.

Procedure for Assay: Weigh accurately about 0.5g of furosemide and dissolve in 40ml of dimethyl formamide. Add few drops of bromothymol blue solution and titrate with 0.1N sodium hydroxide solution. Perform a blank determination and make any necessary correction.

Each ml of 0.1N sodium hydroxide is equivalent to 0.03308g of furosemide.

Assay of Diethylcarbamizine citrate

- **PRINCIPLE:** Diethylcarbamizine citrate is an example of anti-filarial and anti-helmenthic drug. It is assayed by acidimetry and alkalimetry. Aqueous solution of drug is made alkaline with sodium hydroxide solution. The free base liberated is extracted with chloroform and evaporated. The residue is dissolved in excess of standard solution of sulphuric acid (0.1N) and excess is back titrated with 0.1N sodium hydroxide solution using bromocresol green as indicator.
- **PROCEDURE:**
- **Procedure for Standardization:** Standardize the 0.1N sulphuric acid using standard solution of sodium carbonate (0.1N) and methyl orange indicator. Later, standardize the sodium hydroxide solution (0.1 N) using the standardized sulphuric acid and phenolphthalein indicator.
- **Procedure for Assay:** Weigh accurately about 0.5g of diethylcarbamizine citrate and add dissolve in 50ml of water. Add 5ml of 5N sodium hydroxide solution. Add 25ml of 0.1N sulphuric acid and 20ml of water to the residue. Titrate the excess of acid with 0.1N sodium hydroxide using bromocresol green as indicator.
- Each ml of 0.1N sulphuric acid is equivalent to 0.03914g of diethylcarbamizine citrate.

Assay of Phenobarbitone

PRINCIPLE: Phenobarbitone is an example of sedative drug. It is estimated by acid base titration i.e., alkalimetry. In this method, solution of phenobarbitone sodium in sulphuric acid is titrated against standard solution of sodium hydroxide (0.1M) using phenolphthalein as indicator.

PROCEDURE:

- **Procedure for Standardization:** Standardize the 0.1M sodium hydroxide using standard solution of oxalic acid (0.1M) and phenolphthalein indicator.
- **Procedure for Assay:** Weigh accurately about 0.15g of phenobarbitone sodium and dissolve in 2ml of water. Add 8ml of 0.05M sulphuric acid. Heat to boiling and cool. Add 30ml of methanol and titrate with 0.1M sodium hydroxide using phenolphthalein as an indicator.
- Each ml of 0.1M is equivalent to 0.02542g of phenobarbitone sodium.

THANK YOU