6 PERMANGANATE, DICHROMATE AND CERIC SULPHATE TITRATION METHODS

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6.1. INTRODUCTION

The oxidation and reduction processes essentially take place simultaneously in a reaction, thus one entity gets reduced in the process of oxidizing the second. 'Redox'—is the abbreviated form of reduction—oxidation systems. In the **oxidation—reduction methods of analysis** a change in valence of the reacting products is a must which is contrary to precipitation and neutralization methods of analysis where no change in valence occur. The major oxidizing agents normally employed in volumetric titrations include, potassium permanganate, potassium dichromate, and ceric sulphate.

6.2. THEORY

As a number of elements are capable of exhibiting more than one oxidation state, hence volumetric titration methods based on redox reactions are usually employed widely.

The phenomenon of oxidation may be explained in the following manner :

(*i*) addition of oxygen :

Example : $SO_2 + O \longrightarrow SO_3$

(*ii*) removal of hydrogen :

Example : $H_2S + O \longrightarrow H_2O + S$

(*iii*) enhancement in the ratio of electronegative to the electropositive portion of the molecule :

Examples :

(1) $Sn^{2+} + 2Cl^- + 2HgCl_2 \longrightarrow Sn^{4+} + 4Cl^- + Hg_2Cl_2$

(2) $2Fe^{2+} + 4Cl^- + Cl_2 \longrightarrow 2Fe^{3+}\ 6Cl^-$

In the same vein, the process of reduction may also be explained as stated below :

(*i*) addition of hydrogen :

Example : $C_2H_4 + 2H \longrightarrow C_2H_6$

(*ii*) removal of oxygen :

Example : $CuO + 2H \longrightarrow Cu + H_2O$

(*iii*) enhancement in the ratio of electropositive to electronegative portion of the molecule :

Example : [same as under oxidation (*iii*) above]

It is quite evident from the above cited examples that reduction need not always imply a reaction involving hydrogen, since $HgCl_2$ is reduced to Hg_2Cl_2, and that oxidation may not essentially suggest a reaction involving oxygen, since Fe^{2+} is oxidized by Cl_2 to Fe^{3+}. It is, therefore, pertinent to observe here that whenever one entity undergoes oxidation, definitely some other entity undergoes reduction correspondingly and *vice-versa*. In other words, there always exists a transfer of electrons in oxidation-reduction reactions, because in every such reaction the charge gained or lost by one substance must essentially be lost or gained by another.

A **reducing agent** is the reactant that loses electrons in an oxidation-reduction reaction :

$$Fe^{2+} \longrightarrow Fe^{3+} + e$$

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

Thus, the reactant containing a constituent atom or atoms are converted to a higher state of oxidation.

An oxidizing agent is the reactant that gains electrons in an oxidation-reduction reaction :

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

$$Fe^{3+} + e^- \longrightarrow Fe^{2+}$$

Thus, the reactant containing a constituent atom or atoms are converted to a lower state of oxidation.

The quantitative measurement of one of the reactants may be accomplished by the reaction derived from the combination of oxidizing and reducing agents, for instance

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

and hence, ferrous sulphate can be estimated quantitatively by its reaction with ceric sulphate.

6.3. ASSAY METHODS

The quantitative estimations of a number of pharmaceutical substances may be carried out by using a variety of potential oxidizing agents as stated below :

(*i*) **Permanganate Methods :**

(*a*) Direct Titration Methods,

(*b*) Indirect Titration Methods, and

(*c*) Residual Titration Methods.

(*ii*) **Dichromate Methods :**

Direct titrations with Potassium Dichromate.

(*iii*) **Ceric Sulphate Titration Methods :**

Direct Titrations with Ceric Sulphate

6.3.1. PERMANGANATE METHODS

The vital application of potassium permanganate as a potential oxidizing agent in an acidic medium mainly rests on the reactions designated by the following equations :

Chemically we have :

$$2KMnO_4 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 3H_2O + 5(O)$$

Ionically we have :

$$MnO_4^- + 8H^+ + 5e \longrightarrow Mn^{2+} + 4\ H_2O$$

Therefore, $KMnO_4 \equiv 5e$

or 158.0 g $KMnO_4 \equiv$ 5000 ml N

or 31.60 g $KMnO_4 \equiv$ 1000 ml N

or 3.16 g $KMnO_4 \equiv$ 1000 ml 0.1 N $KMnO_4$

6.3.1.1. Preparation of 0.1 N Potassium Permanganate Solution

Materials Required : Potassium permanganate : 3.5 g.

Procedure : Weigh accurately about 3.2 g of potassium permanganate on a watch-glass. Transfer the contents to a 250 ml beaker containing cold water and stir vigorously with a glass rod to effect rapid dissolution. Decant the solution through a small plug of glass wool supported by a funnel, into a 1 litre volumetric flask thereby leaving the undissolved residues in the beaker. Add more DW to the beaker and repeat the above process till all the potassium permanganate gets dissolved. Finally make up the volume to the graduated mark and shake well so as to effect uniform mixing.

Note : (*i*) $KMnO_4$ must be weighed on a watch-glass and not on any kind of paper since cellulose fibers are corrosively attacked by it,

(*ii*) Likewise, filtration of $KMnO_4$ solution must be done though cleaned glass wool and not cotton wool, and

(*iii*) Avoid heat in the preparation of $KMnO_4$ solution because traces of grease or other possible contaminants on the glass vessels used can catalyse its decomposition.

6.3.1.2. Standardization of 0.1 N Potassium Permanganate Solution

Materials Required : Oxalic acid : 6.3 g ; sulphuric acid concentrated : 5 ml.

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

$$\begin{matrix} COOH \\ | \\ COOH \end{matrix} \longrightarrow 2CO_2 + 2H^+ + 2e$$

Therefore, $H_2C_2O_4.2H_2O \equiv 2e$

or 126.04 g $H_2C_2O_4.2H_2O \equiv$ 2000 ml N

or 63.02 g $H_2C_2H_2.2H_2O \equiv$ 1000 ml N

or 6.302 $H_2C_2O_2.2H_2O \equiv$ 1000 ml 0.1 N $KMnO_4$

Procedure : Weigh accurately about 6.3 g of pure oxalic acid (AnalaR-Grade) into a 1 litre volumetric flask, dissolve in suffcient DW and make up the volume upto the mark. Pipette out 25 ml of this solution, add to it 5 ml of concentrated sulphuric acid along the side of the flask, swirl the contents carefully and warm upto 70°C. Titrate this against the potassium permanganate solution from the burette till the pink colour persists for about 20 seconds.

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.

6.3.1.3. Direct Titration Methods

Hydrogen peroxide solution and potassium bromide are two pharmaceutical substances that may be estimated by employing 0.1 N potassium permanganate solution and adopting the direct titration method.

6.3.1.3.1. Hydrogen Peroxide Solution

Materials Required : Hydrogen peroxide solution : 10 ml ; 5 N sulphuric acid : 5 ml ; 0.1 N potassium permanganate.

Procedure : Dilute 10 ml of hydrogen peroxide solution to 250 ml with DW in a volumetric flask. To 25.0 ml of this solution add 5 ml of 5 N sulphuric acid and titrate with 0.1 N $KMnO_4$ to a permanent pink endpoint. Each ml of 0.1 N potassium permanganate is equivalent to 0.001701 g of H_2O_2.

Equations :

Chemically, we have :

$$2KMnO_4 + 3H_2SO_4 \longrightarrow K_2SO + 2MnSO_4 + 3H_2O + 5(O)$$

$$5H_2O_2 + 5(O) \longrightarrow 5O_2 + 5H_2O$$

Summing up : $5H_2O_2 + 2KMnO_4 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2 \uparrow$

Ionically we have :

$$2MnO^-_4 + 6H^+ + 5H_2O_2 \longrightarrow 2Mn^{2+} + 8H_2O + 5O_2 \uparrow$$

Therefore, $5H_2O_2 \equiv 2MnO_4^- \equiv$ l0e

or 34.02 g $H_2O_2 \equiv 2000$ ml N

or 17.01 g $H_2O_2 \equiv 1000$ ml N

or 0.001701 g $H_2O_2 \equiv 1$ ml 0.1 N $KMnO_4$

Calculations : (For % w/v of H_2O_2)

The 'volume strength' of the hydrogen peroxide solution is the number of ml of oxygen at NTP* which may be produced by the complete thermal decomposition of 1 ml of H_2O_2 solution. Hence, decomposition takes place as designated by the following equation :

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

or 68.04 g $H_2O_2 \equiv 22400$ **ml O_2

or 1 g $H_2O_2 \equiv 329.2$ ml O_2

The IP limit of H_2O_2 solution is 5-7% w/v.

Now, let us consider a sample which contains 6.25 per cent w/v H_2O_2 :

Therefore, 100 ml sample $\equiv 6.25$ g H_2O_2

or 1 ml sample $\equiv 0.0625$ g H_2O_2

$\equiv 0.0625 \times 329.2$ ml O_2

$\equiv 20.58$ ml O_2

Hence, the volume strength of the sample is 20.58.

6.3.1.3.2. *Potassium Bromide*

Materials Required : Potassium bromide : 1.2 g ; sulphuric acid (36 N) ; 10 ml ; 0.1 N $KMnO_4$.

* NTP = Normal temperature and pressure.

** At standard temperature and pressure (STP) 1 mole of $O_2 \equiv 22.4$ L.

Procedure : Weigh accurately about 1.2 g of potassium bromide and dissolve in DW and make up the volume to 1 litre mark with water in a volumetric flask. To 10.0 ml of the solution, add 100 ml of DW and 10 ml of (36 N) sulphuric acid along the side of the flask and a few glass beads (to avoid bumping of solution). Heat to boiling and while the solution is still boiling, titrate with 0.1 N $KMnO_4$ added dropwise until the pink colour just persists. Each ml of 0.1 N $KMnO_4$ is equivalent to 0.01190 g of KBr.

Equations :

The Br^- is oxidised to bromine by acidified $KMnO_4$, thus :

$$2Br^- \longrightarrow Br_2 + 2e$$

or $2KBr \equiv 2e$

or 119 g KBr ≡ 1000 ml N $KMnO_4$

or 0.0119 g KBr ≡ 1 ml 0.1 N $KMnO_4$

6.3.1.4. Indirect Titration Methods

In the indirect method of permanganate oxidation certain compounds are first converted by means of chemical reactions to an equivalent amount of oxalate which is then subsequently oxidized quantitatively by permanganate.

6.3.1.4.1. *Assay of Cherry Juice for Malic Acid*

In this particular assay the malic acid present in the cherry juice is estimated by the following *three* steps sequentially :

Step 1 : Conversion of malic acid to an equivalent amount of calcium salt,

Step 2 : Conversion of calcium salt to corresponding insoluble calcium oxalate, and

Step 3 : Liberation of oxalate and subsequent oxidation with permanganate.

Materials Required : Cherry juice : 10 ml ; calcium carbonate : 1.0 g ; ammonia TS : 1 ml ; ammonium oxalate TS : 15 ml ; diluted ammonia (1 in 49) : 25 ml ; diluted sulphuric acid (1 in 3 ; approximately 9 N) : 30 ml ; potassium permanganate 0.1 N.

Procedure : Place 10 ml of precisely measured cherry juice in a 125 ml flask and add to it 1 g of calcium carbonate. Heat the contents on a water-bath for 15 minutes while swirling periodically and filter. Wash the filter 2 to 3 times with 5 ml portions of DW. Add to the combined filtrate and washings 1 ml of ammonia TS followed by 15 ml of ammonium oxalate TS. Warm the contents on a water-bath for 15 minutes, filter through filter paper and wash the filter with 5 ml portions of a solution previously made by mixing 1 ml of ammonia TS with 49 ml of DW. Perforate the filter paper and wash the precipitate into the same flask with hot DW and followed by 30 ml of diluted sulphuric acid. The resulting solution is heated to 80°C and finally titrated with 0.1 N $KMnO_4$. Each ml of 0.1 N $KMnO_4$ is equivalent to 6.704 g of $C_4H_6O_5$.

Equations : Malic acid first reacts with $CaCO_3$ to yield the soluble calcium malate that goes into the filtrate, whereas the insoluble calcium carbonate is filtered off and rejected. Thus,

$$\underset{\text{Malic acid}}{C_4H_6O_5} + CaCO_3 \longrightarrow \underset{\substack{\text{Calcium malate}\\\text{(soluble)}}}{CaC_4H_4O_5} + CO_2\uparrow + H_2O$$

The interaction between calcium malate and ammonium oxalate results into an equivalent quantity of calcium oxalate by displacement mechanism which is subsequently precipitated :

$$\underset{\text{Calcium malate}}{CaC_4H_4O_5} + \underset{\text{Ammonium oxalate}}{(NH_4)_2C_2O_4} \longrightarrow \underset{\text{Calcium oxalate}}{CaC_2O_4 \cdot H_2O}\downarrow + \underset{\text{Ammonium malate}}{(NH_4)_2C_4H_6O_5}$$

Calculations :

Therefore, $C_4H_6O_5 \equiv CaC_2O_4.H_2O \equiv 2000$ ml N $KMnO_4$

or 134.08 g $C_4H_6O_5 \equiv 2000$ ml N $KMnO_4$

or 67.04 g $C_4H_6O_5 \equiv 1000$ ml N $KMnO_4$

or 0.06704 g $C_4H_6O_5 \equiv 1$ ml 0.1 N $KMnO_4$

6.3.1.5. Residual Titration Methods

The residual titration method for pharmaceutical substances using potassium permanganate solution are mainly of *two* categories, namely :

(*i*) titration wherein an excess of standard oxalic acid is added to the substance and then the excess of oxalic acid is back titrated with $KMnO_4$, and

(*ii*) titration wherein an excess of standard $KMnO_4$ solution is used to oxidize the product, and then the amount in excess is estimated by reduction with either :

(*a*) excess ferrous ammonium sulphate and back titrated with more of standard $KMnO_4$, or

(*b*) excess standard oxalic acid.

6.3.1.5A. *Assay of Sodium Nitrite*

Materials Required : Sodium nitrite : 1.0 g ; 0.1 N potassium permanganate : 50 ml ; sulphuric acid (conc.) : 5 ml ; 0.1 N oxalic acid.

Procedure : Weigh accurately about 1 g of sodium nitrite and dissolve it in DW to make 100 ml in a volumetric flask. Transfer 10 ml of this solution into a mixture of 50 ml of 0.1 N $KMnO_4$, 100 ml of water and add 5 ml of sulphuric acid along the side of the flask. Heat the contents to 40°C, allow it to stand for 5 minutes and add 25 ml of 0.1 N oxalic acid. Warm the resulting mixture to about 80°C on a steam-bath and titrate with 0.1 N $KMnO_4$ solution. Each ml of 0.1 N potassium permanganate is equivalent to 3.450 mg of $NaNO_2$.

Precautions : While adding $NaNO_2$ solution

(*i*) Care should be taken to immerse the tip of the pipette beneath the surface of the permanganate mixture, otherwise the nitrous acid (volatile) generated by $NaNO_2$ and H_2SO_4, would be lost, and

(*ii*) Oxidation of nitrous acid (HNO_2) to nitric acid (HNO_3) takes place sluggishly at ambient temperature and hence, it is necessary to warm it upto 40°C for 5 minutes to expedite completion of reaction.

Equations : Chemically we have :

$$10NaNO_2 + 4\ KMnO_4 + 11H_2SO_4 \longrightarrow 10HNO_3 + 4MnSO_4 + 2K_2SO_4 + 5Na_2SO_4 + 6H_2O$$

(69.0)

Ionically we have :

$$NO_2^- + HO \longrightarrow NO_3^- + 2H^+ + 2e$$

i.e., each molecule of sodium nitrite loses two electrons.

Calculations :

Therefore, $NaNO_2 \equiv NO_2^- \equiv 2e$

or 69.0 g $NaNO_2 \equiv 2000$ ml N

or 0.003450 g $NaNO_2 \equiv 1$ ml of 0.1 N $KMnO_4$

6.3.2. DICHROMATE METHODS

Potassium dischromate ($K_2Cr_2O_7$) is a strong oxidizing agent, quite comparable to $KMnO_4$ that normally shows only one pertinent reduced oxidation state : Thus, chemically we have :

$$K_2Cr_2O_7 + 4H_2SO_4 \longrightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3(O)$$

Ionically we have :

$$K_2Cr_2O_7 + 14H^+ + 6e^- \longrightarrow 2K^+ + 2Cr^{3+} + 7H_2O$$

Therefore, we have :

$$K_2Cr_2O_7 \equiv 6e$$

or $$294.0 \text{ g } K_2Cr_2O_7 \equiv 6000 \text{ ml N}$$

or $$49.0 \text{ g } K_2Cr_2O_7 \equiv 1000 \text{ ml N}$$

or $$4.90 \text{ g } K_2Cr_2O_7 \equiv 100 \text{ ml of 0.1 N } K_2Cr_2O_7$$

Potassium dichromate exhibits much greater stability in aqueous solution in comparison to potassium permanganate. Potassium dichromate possesses an inherent oranage colour that is not intense enough to serve its own end-point signal, specifically in the presence of the green Cr^{3+} ion, which is supposed to be present at the end-point. Hence, redox indicators are usually employed to locate the exact end-point *e.g.*, barium diphenylamine sulphonate.

6.3.2.1. Preparation of 0.1 N Potassium Dichromate Solution

Materials Required : Potassium dichromate : 4.930 g.

Procedure : Weigh accurately 4.93 g of potassium dichromate previously powdered and dried at 20°C for 4 hours and dissolve in sufficient DW to produce 1 litre in a volumetric flask.

Note : Potassium dichromate can be obtained as a primary standard reagent and hence, standard solutions may be prepared determinately and stored for long periods of time.

Equations : Chemically we have :

$$K_2Cr_2O_7 + 4H_2SO_4 \longrightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3(O)$$

Ionically we have :

$$K_2Cr_2O_7 + 14H^+ + 6e^- \longrightarrow 2K^+ + 2Cr^{3+} + 7H_2O$$

From this equation it follows that the equivalent weight of potassium dichromate is 1/6th of the molecular weight *i.e.*, 294.22/6 or 49.03 g.

6.3.2.2. Standardization of 0.1 N Potassium Dichromate Solution

It can be achieved by following these steps, namely :

(*a*) **Preparation of Standard Solution of Mohr's Salt $FeSO_4(NH_4)_2.SO_4.6H_2O$:**

Materials Required : Mohr's salt : 4.9 g ; dilute sulphuric acid (1 in 3, approx. 9 N) : 20 ml.

Procedure : Weigh accurately about 4.9 g of pure sample of Mohr's salt and transfer it to a 250 ml volumetric flask. Add 20 ml of dilute sulphuric acid and make up the volume to the mark with DW and finally mix the contents of the flask thoroughly.

Calculations : The quantity of Mohr's salt required for 250 ml of the solution having a normality of 0.05 N can be calculated as follows :

$$\text{Mohr's salt} = \frac{\text{Eq. wt. of Mohr's salt} \times \text{Volume}}{1000} \times 0.05$$

or $$= 4.9 \text{ g}$$

(*b*) **Standardization of 0.1 N $K_2Cr_2O_7$ Solution :**

Materials Required : Standard solution of Mohr's salt (0.05 N) : 250 ml, sulphuric acid (2 N) : 20 ml ; potassium dichromate solution (0.1 N) : 1 litre.

Procedure : Transfer 20 ml of the primary standard solution (Mohr's salt) to the titration flask and add 20 ml of 2 N sulphuric acid. Take the potassium dichromate solution in the burette. Put drops of freshly

prepared potassium ferricyanide, $K_3[Fe(CN)_6]$, solution in the grooves of a porcelain tile. Now, proceed with the titration of Mohr's salt solution against $K_2Cr_2O_7$ solution. Transfer drops of the titrated solution by means of a glass rod and mix with drops of the indicator, already taken in the groove-tile. Alternatively, pre-soaked and dried filter paper with $K_3[Fe(CN)_6]$ solution can also be used in place of the groove-tile method.

In order to arrive at the exact end-point the above titration may be carried out at *three* stages, namely :

Stage 1 : Spot tests are carried out at intervals of 1-2 ml until a blue colour is no longer produced with $K_3[Fe(CN)_6]$, which provides an altogether rough estimate of the $K_2Cr_2O_7$ solution required for the titration,

Stage 2 : Spot tests are only performed near the approach of the end of titration at intervals of 0.1-0.2 ml, and

Stage 3 : Spot tests are finally done only at the end-point.

The above sequential steps give fairly accurate results because the error caused by the removal of part of the solution for the spot tests is made negligibly small. However, the titration is repeated to get a set of concordant readings.

By applying the relationship between N_1V_1 ($K_2Cr_2O_7$) and N_2V_2 (Mohr's salt), the normality of the former may be calculated.

6.3.2.2.1. *Iron Ore*

Materials Required : Iron ore : 0. 1 g ; hydrochloric acid (conc.) : 15 ml ; diphenylamine (1% w/v in conc. H_2SO_4) ; zinc metal (granulated) : 4 g ; ammonium thiocyanate solution (0.1% in water) ; mixture of sulphuric acid and phosphoric acid [dissolve 15 ml of H_2SO_4 (sp. gr. 1.84) in 50 ml of DW, cool and add 15 ml of H_3PO_4 (sp. gr. 1.70) and make the volume to 100 ml with DW] : 25 ml.

Procedure :

(*a*) **Preparation of Standard $K_2Cr_2O_7$ Solution :** Instead of using solutions having definite normality, routine industrial laboratories make use of '*emperical solution*' which is normally expressed in terms of '*titer for the substance determined*'. For this assay, let us prepare an emperical $K_2Cr_2O_7$ solution (250 ml) of such a concentration that 1 ml of the same exactly correspond to 0.0025 g Fe.

Calculations :

1000 ml $K_2Cr_2O_7$ soln. ≡ 0.0025 × 1000 ≡ 2.5 g of Fe

or 250 ml $K_2Cr_2O_7$ soln. ≡ 0.6250 g Fe

By Law of Equivalence, we have :

1 gram-equivalent of $K_2Cr_2O_7$ (49.03 g) ≡ 1 gram-equivalent of Fe (55.85 g)

Hence, $$0.6250 \text{ g Fe} \equiv \frac{0.6250 \times 49.03}{55.85} \equiv 0.5488 \text{ g}$$

Therefore, weigh accurately 0.5488 g of pure $K_2Cr_2O_7$ and transfer it quantitatively into a 250 ml volumetric flask, dissolve in DW, make up the volume and mix thoroughly.

Hence, the '**iron titer**' of this solution is :

$$T\ K_2Cr_2O_7/\ Fe = 0.0025 \text{ g / ml}$$

(*b*) **Preparation of Ore Solution :** Weigh accurately 0.1 g of powdered and dried ore on a clean watch glass and transfer it quantitatively into a 100 ml-volumetric flask. Add 15 ml of concentrated hydrochloric acid, warm the contents of the flask carefully over a sand-bath until most of the dark grains of ore get dissolved completely and only a whitish silica precipitate settles at the bottom of the flask.

(*c*) **Reduction of Fe^{3+} to F^{2+} in the Ore Solution :** Introduce carefully a few pieces of granulated pure zinc metal into the flask, place a funnel in the neck of the flask to avoid splashes and boil the solution gently until the yellow colour has disappeared completely, thereby ascertaining that complete reduction of Fe^{3+} to Fe^{2+} is affected.

Note : It may be further confirmed by doing a spot test with NH_4CNS solution which only shows a blood-red colour with Fe^{3+}.

The contents of the flask is cooled, filtered through cotton wool, washings done with DW and the filtrate diluted to about 350 ml with DW. This dilution is a must so as to avoid any interference caused by its inherent green colour with the estimation of the equivalence point in the titration as per the following chemical reaction :

$$K_2Cr_2O_7 + 6FeCl_2 + 14HCl \longrightarrow 2CrCl_3 + 2KCl + 6FeCl_3 + 7H_2O$$

(*d*) **Final Titration :** The 350 ml solution obtained in (*c*) above is now quantitatively titrated against $K_2Cr_2O_7$ solution employing diphenylamine as an internal indicator. Add 25 ml of a mixture of sulphuric acid and phosphoric acid to the solution along with 2 drops of diphenylamine indicator and titrate the solution with $K_2Cr_2O_7$ solution carefully, by adding small lots at intervals with constant shaking, until a persistant blue-violet colour appears.

Note : (*a*) **The acidity of the solution must be maintained fairly high which can be achieved by adding orthophosphoric acid, H_3PO_4,**

(*b*) **The quantity of diphenylamine must not exceed 2 drops by virtue of the fact that at higher concentration with lower acidity during very slow titration, the indicator undergoes an altogether different type of chemical change that ultimately gives a green colour instead of the desired blue-violet colour.**

(*e*) **Calculations :** Multiply the number of millilitres of $K_2Cr_2O_7$ Solution consumed in the titration by the 'iron titer' and therefrom determine the amount of iron present in the sample. Finally, the percentage of iron present in the ore may be calculated.

6.3.3. CERIC SULPHATE TITRATION METHODS

Ammonium ceric sulphate serves as a powerful oxidizing agent in an acidic medium. The salt has a bright yellow colour and so its solution. On reduction, the resulting cerous salt obtained is colourless in appearance and, therefore, strong solutions may be considered as self-indicating. In general practice, 0.05 N solutions are employed invariably for estimations. As this concentration is very dilute for observation of the respective end-point, hence the inclusion of an appropriate indicator becomes necessary. The oxidation reaction involved may be expressed as follows :

$$Ce^{4+} + e \rightleftharpoons Ce^{3+}$$

It is interesting to observe that the solutions of ammoniurn ceric sulphate possess a number of advantages over permanganate and dichromate methods discussed earlier in this chapter, *viz.*,

(*i*) solutions remain fairly stable even when boiled,

(*ii*) solutions quantitatively react with either arsenite (AsO_3^{3-}) or oxalate $[(COO)_2]^{2-}$ ion, and therefore, either arsenic trioxide or sodium oxalate may be employed as a primary standard,

(*iii*) cerous ion Ce^{3+} is colourless and hence offers no interference with the indicator end-point,

(*iv*) Ce^{3+} always solely results on reduction of Ce^{4+}, whereas permanganate (MaO_4^-) can be reduced to any of several oxidation states,

(*v*) ammonium ceric sulphate unlike potassium permanganate, may be conveniently employed as an oxidizing agent in the presence of high concentrations of HCl, thereby facilitating determinations of Fe^{2+} in the presence of Cl^-, and

(*vi*) ferrous phenanthrolone ion (ferroin) has proved to be a very successful indicator in titrations with ceric salts. Thus, we have :

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

Orthophenanthroline (base) dissolves rapidly in aqueous solutions of ferrous salts, thereby three moles combine with one Fe^{2+} ion to give a complex termed as '**ferroin**' having an, intense red colour. Now, any strong oxidizing agent converts the ferrous to a corresponding ferric complex having a slight blue colour.

6.3.3.1. Preparation of 0.1 N Ammonium Ceric Sulphate Solution

Materials Required : Ceric ammonium sulphate : 66 g ; sulphuric acid (conc.) : 30 ml.

Procedure : Dissolve 66 g of ceric ammonium sulphate, with the help of gentle heat, in a mixture of 30 ml of sulphuric acid and 500 ml DW. Cool, filter the solution through a fine-porosity sintered-glass crucible, dilute to 1 litre mark in a volumetric flask and mix thoroughly.

Since the oxidation reaction is given by :

$$Ce^{4+} + e \rightleftharpoons Ce^{3+}$$

Therefore, 632.57 g $Ce(SO_4)_2\ 2(NH_4)_2SO_4.2H_2O \equiv$ 1000 ml N

or 63.26 g $Ce(SO_4)_2\ 2(NH_4)_2\ SO_4.2H_2O \equiv$ 1000 ml 0.1 N ammonium ceric sulphate

6.3.3.2. Stadardization of 0.1 N Ammonium Ceric Sulphate Solution

Materials Required : Arsenic trioxide : 0.2 g ; sodium hydroxide solution (8.0% w/v) : 25 ml ; diluted sulphuric acid (10% w/v) : 30 ml ; osmic acid solution (1.0% w/v in water) : 0.15 ml ; ferroin sulphate solution (dissolve 0.7 g of ferrous sulphate in 70 ml of DW and add 1.5 g of 1, 10-phenanthroline and sufficient water to produce 100 ml) : 0.1 ml.

Procedure : 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. Wash down the inner walls of the flask with 25 ml of sodium hydroxide solution, swirl to dissolve, add 100 ml of water and mix. Add 30 ml of diluted sulphuric acid, 0.15 ml of osmic acid solution, 0.1 ml of ferroin sulphate solution and slowly titrate with ceric ammonium sulphate solution until the pink colour is changed to a very pale blue. Each 4.946 mg of arsenic trioxide is equivalent to 1 ml of 0.1 N ammonium ceric sulphate or 0.06326 g of $Ce(SO_4)_2.\ 2(NH_4)_2SO_4\ .\ 2H_2O$.

Equations :

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

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

It is evident from the above equations that 4 equivalents of ceric sulphate is required to oxidise 1 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 milliequivalent shall contain 49.46 mg or 0.04946 g.

Calculations : Therefore, the normality of ammonium ceric sulphate solution may be expressed as follows :

$$N = \frac{\text{wt. of arsenic trioxide}}{\text{ml} \times 0.04946}$$

6.3.3.2.1. *Ferrous Fumarate*

Materials Required : Ferrous fumarate : 0.3 g ; diluted H_2SO_4 (10% w/v) : 15 ml ; ferroin sulphate solution ; 0.1 N ammonium ceric sulphate solution.

Procedure : Weigh accurately about 0.3 g of ferrous fumarate and dissolve in 15 ml of dilute sulphuric acid by the help of gentle heating. Cool, add 50 ml of water and titrate immediately with 0.1 N ammonium ceric sulphate, employing ferroin sulphate solution as indicator. Each ml of 0.1 N ammonium ceric sulphate is equivalent to 0.01699 g of $C_4H_2FeO_4$.

Equations and Calculations :

$$C_4H_2FeO_4 \equiv Fe \equiv e$$

Therefore, 169.91 g $C_4H_2FeO_4 \equiv$ 1000 ml N

or 16.99 g $C_4H_2FeO_4 \equiv$ 1000 ml 0.1 N

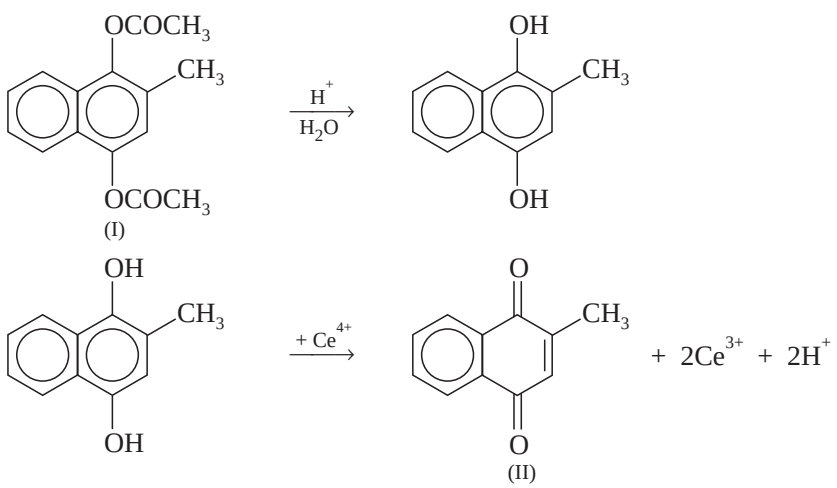
or 0.01699 g $C_4H_2FeO_4 \equiv$ 1 ml 0.1 N Ammonium ceric sulphate

6.3.3.2.2. *Acetomenaphthone*

Materials Required : Acetomenaphthone : 0.2 g ; glacial acetic acid : 15 ml ; dilute hydrochloric acid (10% w/v) : 15 ml ; ammonium ceric sulphate 0.05 N ; ferroin sulphate solution.

Procedure : Weigh accurately about 0.2 g of acetomenaphthone and boil it with 15 ml of glacial acetic acid and 15 ml of dilute hydrochloric acid under a reflux condenser for 15 minutes. Cool the contents carefully and taking adequate precautions to avoid any atmospheric oxidation. Add 0.1 ml of ferroin sulphate solution as indicator and titrate with 0.05 N ammonium ceric sulphate. Repeat the assay without the substance being examined (blank determination) and incorporate the correction, if any. Each ml of 0.05 N ammonium ceric sulphate is equivalent to 0.006457 g of $C_{15}H_{14}O_4$.

Equations :



First, acetamenaphthone (I) undergoes hydrolysis in acidic medium to yield the corresponding phenol and secondly, this phenol is oxidised quantitatively with ammonium ceric sulphate to give the resulting 1, 4-dione derivative (II).

Calculations :

Thus, we have :

$$258.3 \text{ g } C_{15}H_{14}O_4 \equiv 2Ce^{4+} \equiv 2000 \text{ ml N}$$

or 129.15 g $C_{15}H_{14}O_4 \equiv$ 1000 ml N

or 0.12915 g $C_{15}H_{14}O_4 \equiv$ 1 ml N

or 0.012915 g $C_{15}H_{14}O_4 \equiv$ 1 ml 0.1 N

or 0.006457 g $C_{15}H_{14}O_4 \equiv$ 1 ml 0.05 N Ammonium ceric sulphate

6.3.3.2.3. *Cognate Assays*

A number of pharmaceutical substances and dosage forms may be determined by the help of ceric sulphate titration methods as given in Table 6.1.

Table 6.1 : Redox Titrations : Ceric Sulphate Titration Method

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Ferrous Gluconate	1.5 g	Ferroin sulphate	Each ml of 0.1 N ammonium ceric sulphate = 0.04461 g of $C_{12}H_{22}FeO_{14}$
2.	Ferrous sulphate	1.0 g	-do-	Each ml of 0.1 N ammonium ceric sulphate ≡ 0.0278 g of $FeSO_4 . 7H_2O$
3.	Iron Dextran Injection	2.0 g	-do-	Each ml of 0.1 N ammonium ceric sulphate ≡ 0.005585 g of Fe
4.	Menadione	1.5 g	-do-	Each ml of 0.1 N ammonium ceric sulphate ≡ 0.00861 g of $C_{11}H_8O_2$
5.	Paracetamol	0.3 g	-do-	Each ml of 0.1 N ammonium ceric sulphate ≡ 0.00756 g of $C_8H_9NO_2$
6.	Tocopherol acetate	0.3 g	Diphenyl amine	Each ml of 0.1 N ammonium ceric sulphate ≡ 0.002364 g of $C_{31}H_{52}O_3$

THEORETICAL AND PRACTICAL EXERCISES

l. Discuss the various theoretical aspects involved in the assay of **permanganate, dichromate** and **ceric sulphate** titration methods. Give equations to explain your logical stand.

2. The '**permanganate methods**' essentially consist of *three* ways to assay pharmaceutical substances :

(*i*) Direct titration method, (*ii*) Indirect titration method, and

(*iii*) Residual titration method.

Discuss any *ONE* of these methods explicitely with the help of a typical example.

3. Discuss direct titration method using '**dichromate method**' in the assay of '**Iron Ore**' with reference to the following aspects :

(*i*) Preparation of 0.1 N $K_2Cr_2O_7$ solution 1 L

(*ii*) Standardization of 0.1 N $K_2Cr_2O_7$ solution using Mohr's salt.

4. (*a*) Describe the '**direct titration with Ceric Sulphate**' and enumerate its advantages over '*permanganate*' and '*dichromate*' methods.

(*b*) How would you standardize 0.1 N ammonium-ceric sulphate solution ? Explain.

(*c*) Give the details for the assay of the following drugs by the direct titration with ceric sulphate solution :

(*i*) Ferrous Fumarate (*ii*) Iron-Dextran Injection

(*iii*) Paracetamol (*iv*) Tocophenol acctate.

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7 IODIMETRIC AND IODOMETRIC TITRATIONS

CONTAINS :

7.1 Introduction
7.2 Theory
7.3 Assay Methods
7.3.1 Iodimetric assays
7.3.2 Iodometric assays

7.1. INTRODUCTION

Iodimetric and iodometric titrations constitute another class of oxidation-reduction titrations wherein either iodine solutions are employed directly for the assay or an equivalent amount of iodine is liberated indirectly from the reaction mixture and then assayed.

Iodimetry is a procedure based on the following reversible reaction :

$$2I^- \rightleftharpoons I_2 + 2e$$

Hence, it can be utilized for the quantitative estimation of reducing agents like arsenites (H_3AsO_3) and thiosulphates ($Na_2S_2O_3$) by employing a standard solution of iodine.

Iodometry is an indirect procedure based on the aforesaid reversible reaction whereby the assay of oxidizing agents, for instance : 'available chlorine' in bleaching powder, cupric and ferric salts may be carried out by reducing them with an excess potassium iodide thereby liberating an equivalent quantity of iodine which can be estimated using a standard solution of thiosulphate.

7.2. THEORY

In **iodimetry**, quantitative oxidation of reducing agents, such as arsenious acid (H_2AsO_3) may be carried out by employing standard solutions of iodine as shown under :

$$H_3AsO_3 + H_2O + I_2 \rightleftharpoons H_3AsO_4 + 2H^+ + 2I^-$$

This type of assay is known as **'direct method of iodimetry'.**

In another situation, a known excess quantity of standard iodine solution is added in the substance (a reducing agent) to be assayed and then the excess iodine may be titrated with the help of standard sodium thiosulphate solution, such as : the estimation of sodium bisulphite :

$$NaHSO_3 + I_2 + H_2O \longrightarrow NaHSO_4 + 2HI$$

$$I_2 + \underset{\text{Sodium thiosulphate}}{2Na_2S_2O_3} \longrightarrow 2NaI + \underset{\text{Sodium tetrathionate}}{Na_2S_4O_6}$$

This category of assay is termed as '**residual method of iodimetry**'.

In **iodometry**, an equivalent amount of iodine is liberated when the given sample of an oxidizing agent oxidizes potassium iodide in an acidic medium, for example : the determination of cupric sulphate ($CuSO_4$) :

$$2CuSO_4 + 4\,KI \longrightarrow 2CuI \downarrow + I_2 + 2K_2SO_4$$

Consequently, the equivalent amount of iodine generated by the above reaction may be conveniently assayed by titration against a standard sodium thiosulphate solution. In this context a point of caution must be observed while KI is being oxidized under a strongly acidic medium so as to avoid simultaneous oxidation of the iodide by atmospheric oxygen that may result high erroneous titer values leading to false estimations.

It is, however, pertinent to mention here that iodometric assays are never performed in a strongly basic medium, because of the fact that the reaction between I_2 and OH^- produces hypoiodide and iodate ions respectively as shown below :

$$I_2 + OH^- \longrightarrow HI + IO^-$$
$$3IO^- \longrightarrow IO_3^- + 2I$$

The said two ions partially oxidize thiosulphate to a higher oxidation form, such as sulphate (SO_4^{2-}) thereby the stoichiometry achieved is always false.

7.3. ASSAY METHODS

Assay methods involving iodine can be categorized under the following heads namely :

A. Iodimetric Assays :

(*a*) Direct titration with iodine,

(*b*) Residual titration method : *i.e.*, excess of iodine is titrated with sodium thiosuphate,

B. Iodometric Assays : *i.e.*, release of iodine and subsequent titration with sodium thiosulphate.

7.3.1. IODIMETRIC ASSAYS

In such estimations, the pharmaceutical substances can be measured either directly or back titration of excess iodine with sodium thiosulphate solution.

7.3.1.1. Direct Titration with Iodine

(*a*) **Preparation of 0.1 Iodine Solution**

Theory : Iodine in aqueous solution acts as an oxidizing agent which forms the basis of assay methods involving direct titration with iodine. Thus, we have :

$$I_2 + 2e \longrightarrow 2I^-$$

or $$I_2 \equiv 2e$$

or $$126.9\text{ g } I_2 \equiv 1000\text{ ml N}$$

or $$12.69\text{ g } I_2 \equiv 1000\text{ ml 0.1 N}$$

or $$3.17\text{ g } I_2 \equiv 250\text{ ml 0.1 N}$$

Materials Required : Iodine : 3.2 g ; potassium iodide : 7.5 g.

Procedure : Weigh accurately 3.2 g of crushed iodine crystals on a watch glass and transfer to a beaker containing potassium iodide (7.5 g) and water (10 ml). Dissolve the contents of the beaker with the help of a glass rod and frequent swirling. Transfer the contents of the beaker quantitatively to a 250 ml volumetric flask and make up the volume with DW.

Explanation : Iodine is sparingly soluble in water but undergoes rapid dissolution in the presence of potassium iodide due to the formation of the corresponding triiodide ion :

$$I_2 + I^- \longrightarrow I_3^-$$

Thus, potassium iodide plays dual role, *viz.*, in iodimetry—to solubilize iodine in aqueous KI solution, and in iodometry—as reducing agent, the excess KI helps in retaining liberated I_2 in solution through interaction with KI.

(*b*) **Standardization of 0.1 Iodine Solution with the aid of Arsenic Trioxide (As_2O_3)**

Theory : This particular standardization is solely governed by the following equations, namely :

$$As_2O_3 + 2H_2O \longrightarrow As_2O_5 + 4H^+ + 4e$$

or $$As_2O_3 \equiv 4e$$

or $$197.8 \text{ g } As_2O_3 \equiv 4000 \text{ ml N}$$

or $$49.45 \text{ g } As_2O_3 \equiv 1000 \text{ ml N}$$

or $$0.4945 \text{ g } As_2O_3 \equiv 100 \text{ ml 0.1 N Iodine}$$

Hydroiodic acid (HI) possesses strong reducing characteristics which renders the oxidation with iodine into a reversible reaction as follows :

$$As_2O_3 + 2I_2 + 2H_2O \rightleftharpoons As_2O_5 + 4H^+ + 4I^-$$

In order to shift the equilibrium to the right-hand-side (*i.e.*, towards As_2O_5) in the above reaction, sodium bicarbonate ($NaHCO_3$) is employed to remove the HI generated. It is important to record here that neither sodium hydroxide nor sodium carbonate can be used as both of them produce sodium iodide (NaI) and sodium iodate ($NaIO_3$) as designated below :

$$6NaOH + 3I_2 \longrightarrow 5NaI + NaIO_3 + 3H_2O$$

$$3Na_2CO_3 + I_2 \longrightarrow 5NaI + NaIO_2 + 3CO_2$$

Materials Required : Arsenic trioxide : 0.5 g ; sodium hydroxide solution (20% w/v in water) : 2 ml ; dilute hydrochloric acid (2N) ; sodium bicarbonate : 4 g ; 0.1 N iodine solution.

Procedure : Weigh accurately 0.5 g arsenic trioxide into a beaker, add to it 2 ml of sodium hydroxide solution, and heat to dissolve. Cool and transfer the contents quantitatively to a 100 ml volumetric flask and make up the volume upto the mark with DW. Pipette 20 ml into an iodine-flask, acidify with dilute HCl carefully and confirm it by adding a little $NaHCO_3$ to remove the free excess acid, followed by a further 2 g to get rid of HI formed in the reaction mixture. Now, titrate with 0.1 N iodine solution till the end-point is achieved by the appearance of the first permanent pale straw colour.

(*c*) **Standardization of 0.1 Iodine Solution by the aid of Sodium Thiosulphate**

Theory : Iodine solution may also be standardized by using sodium thiosulphate (AR-Grade) whereby the latter gets oxidized to sodium tetrathionate as expressed below :

$$2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2e$$

or $$I_2 + 2e^- \longrightarrow 2I^-$$

or $$2S_2O_3^{2-} \equiv I_2 \equiv 2e$$

or $$2 \times 248.2 \text{ g } Na_2S_2O_3.5H_2O \equiv 2000 \text{ ml N}$$

or $$248.2 \text{ g } Na_2S_2O_3.5H_2O \equiv 1000 \text{ ml N}$$

or $$24.82 \text{ g } Na_2S_2O_3.5H_2O \equiv 1000 \text{ ml 0.1 N Iodine}$$

Materials Required : Sodium thiosulphate (AR) : 6.025 g ; 0.1 N I_2 solution.

Procedure : Weigh accurately 6.025 g of sodium thiosulphate (AR) to a 250 ml volumetric flask. Dissolve it in DW, shake well and make up the volume to the mark with DW. Pipette 25 ml of 0.1 iodine

solution into an iodine flask and titrate with the standard sodium thiosulphate solution (as primary standard) until the solution becomes almost colourless.

Note : Stock solutions of sodium thiosulphate may be preserved by the addition of a few drops of sodium hydroxide solution (20% w/v) which serves as stabilizer as well as prevents decomposition.

(*d*) **Preparation of Starch Solution**

Material Required : Starch (arrowroot) : 1.0 g.

Procedure : Weigh 1.0 g starch in a glass in a glass pestle-mortar and triturate thoroughly with 10 ml of cold DW. Boil separately 200 ml of DW in a beaker and add the starch paste to it with vigorous stirring. The resulting mixture is boiled gently for a further period of 30 minutes till a transluscent and thin liquid having an uniform consistency is obtained.

Note : (1) The prepared solution of starch undergoes rapid deterioration, hence it is always desired to use freshly prepared solution every day,

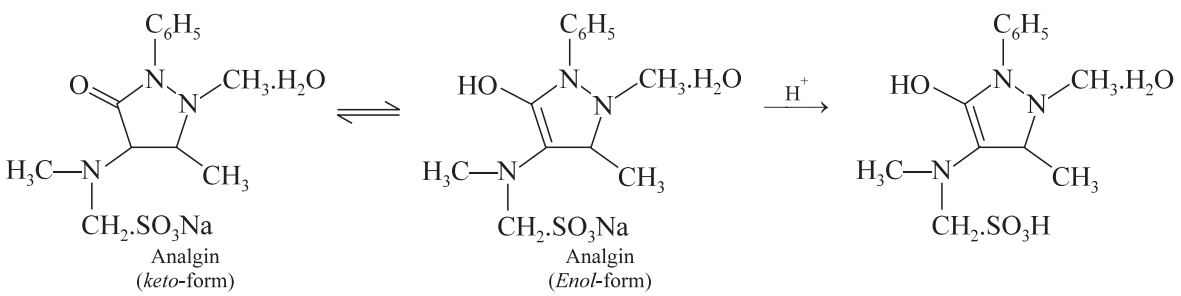
(2) It is now more or less believed that the iodine is held as an 'absorption complex' within the helical chain of the macromolecule β-amylose *i.e.*, a component of most starches. However, another component, α-amylose, is undesirable because it produces a red-colouration with iodine which is not readily reversible, and

(3) 'Soluble Starch' comprises principally of β-amylose, with the α-fraction having been removed. Always, it is a practice to prepare indicator-solutions from this product exclusively.

7.3.1.1.1. *Analgin*

Materials Required : Analgin : 0.4 g ; alcohol (95%) : 40 ml ; 0.01 N hydrochloric acid : 10 ml ; 0.1 N iodine solution.

Theory : The estimation of analgin depends upon the oxidation of the enolic group with iodine. The reaction is not reversible :



Hence, $C_{13}H_{l6}N_3NaO_4S \equiv I_2 \equiv 2e$

or $333.40\ g\ C_{13}H_{16}N_3\ NaO_4S \equiv 2000\ ml\ N$

or $166.70\ g\ C_{13}H_{16}N_3NaO_4S \equiv 1000\ ml\ N$

or $0.01667\ g\ C_{13}H_{16}N_3NaO_4S \equiv 1\ ml\ 0.1\ N\ I_2$

Procedure : Weigh accurately about 0.4 g and dissolve in a mixture of 40 ml of alcohol and 10 ml of 0.01 N hydrochloric acid. Titrate the resulting mixture with 0.1 N iodine solution till a yellow colour that

remains stable for 30 seconds is achieved. Each ml of 0.1 N iodine is equivalent to 0.016670 g of $C_{13}H_{16}N_3NaO_4S$.

7.3.1.1.2. *Acetarsol*

Materials Required : Acetarsol : 0.25 g ; sulphuric acid (conc.) : 7.5 ml ; nitric acid (fuming) : 2.5 ml ; ammonium sulphate : 5 g ; potassium iodide : 1.0 g ; sodium sulphite (0.1 N) : 1.0 ml ; phenolphthalein solution : 2 drops ; NaOH solution (0.1 N) ; dilute sulphuric acid (6 N) ; sodium bicarbonate : 8.0 g ; iodine solution (0.1 N).

Theory : Acetarsol is an organic arsenal, hence arsenic may be estimated by carrying out the oxidation As^{3+} to As^{5+} state with the help of 0.1 N iodine solution.

The organic entity present in acetarsol is destroyed primarily by boiling it with aqua-regia (a mixture of conc. H_2SO_4 and fuming nitric acid). The resulting mixture is heated in the presence of ammonium sulphate to get rid of nitric acid finally in the form of nitrous oxide (N_2O) as follows :

$$2HNO_3 + (NH_4)_2SO_4 \longrightarrow 2NH_4NO_3 + H_2SO_4$$

$$2NH_4NO_3 \xrightarrow{\text{Heat}} 2N_2O \uparrow + 4H_2O$$

Previously added H_2SO_4 maintains an acidic medium which on adding KI liberates HI that reduces the As^{5+} to As^{3+} state. Reduction is completed by boiling the solution which also expels the liberated I_2 as shown below :

$$As_2O_5 + 4HI \longrightarrow As_2O_3 + 2I_2 \uparrow + 2H_2O$$

The resulting mixture is cooled to room temperature and the residual iodine is removed by titration with 0.1 N sodium sulphite solution. Now, the solution is treated with sodium hydroxide solution to make it alkaline and then acidified carefully with dilute H_2SO_4 to remove the free NaOH. Finally, the resulting solution is made alkaline with $NaHCO_3$ so that the equilibrium is shifted to the right (*i.e.*, AS^{3+} gets converted to As^{5+}) quantitatively on carrying out the titration with 0.1 N iodine solution. Thus, we have :

$$As_2O_5 + 2I_2 + 2H_2O \rightleftharpoons As_2O_5 + 4HI$$

or $2C_8H_{10}AsNO_5 \equiv As_2O_5 \equiv As_2O_3 = 2I_2 \equiv 4e$

or 2×275.1 g $C_8H_{10}AsNO_5 \equiv 4000$ ml N

or 550.2 g $C_8H_{10}AsNO_5 \equiv 4000$ ml N

or 137.55 g $C_8H_{10}AsNO_5 \equiv 1000$ ml N

or 0.01375 g $C_8H_{10}AsNO_5 \equiv 1$ ml 0.1 N Iodine

Procedure : Weigh accurately about 0.25 g of acetarsol into a 500 ml iodine flask and add to it sulphuric acid (conc.) 7.5 ml, followed by nitric acid (fuming) 1.5 ml. Boil the contents of the flask gently for 45 minutes preferably in a fume-cupboard. Cool the solution, add 0.5 ml of fuming HNO_3 and boil till brown vapours (N_2O) stop coming. Again cool the contents and add carefully 5 g of ammonium sulphate in small lots at intervals and heat till there is no evolution of N_2O thereby giving rise to a colourless liquid. Bring the solution to room temperature, dilute with 100 ml DW, add 1 g KI and heat gently till the volume becomes 50 ml. Cool and add a few drops of 0.1 N sodium sulphite to effect decolourisation. Add 60 ml DW to dilute the resulting contents and make it just alkaline with NaOH solution by adding phenolphthalein indicator. Finally, acidify with dilute H_2SO_4, neutralize with $NaHCO_3$ and add 4 g of $NaHCO_3$ in excess. Swirl the contents of the flask and titrate with 0.1 N iodine solution. Each ml of 0.1 N iodine solution is equivalent to 0.01375 g of $C_8H_{10}AsNO_5$.

Note : All boiling is to be done in a fume-cupboard.

7.3.1.1.3. *Cognate Assays*

The following pharmaceutical substances can be assayed by direct titration with iodine as stated in Table 7.1.

Table 7.1 : Substances Assayed by Direct Titration with Iodine

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Ascorbic acid	0.1 g	Starch solution	Each ml of 0.1 N Iodine ≡ 0.008806 g of $C_6H_8O_6$
2.	Sodium ascorbate	0.4 g	-do-	Each ml of 0.1 Iodine ≡ 0.009905 g of $C_6H_7NaO_6$
3.	Sodium thiosulphate	0.8 g	-do-	Each ml of 0.1 N Iodine ≡ 0.02482 g of $Na_2S_2O_3.5H_2O$

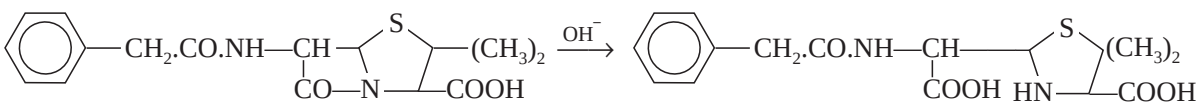
7.3.1.2. Residual Titration Method (*Excess of Iodine Titrated with Sodium Thiosulphate*)

In this titration method an excess of iodine solution is added to the solution of the substance and thus, the latter gets oxidized quantitatively. The excess of iodine is subsequently back titrated with sodium thiosulphate using freshly prepared starch solution as indicator with an end-point from violet to colourless.

7.3.1.2.1. *Benzylpenicillin*

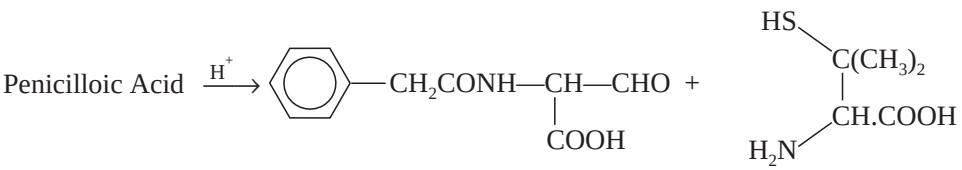
Theory : Benzylpenicillin can be assayed efficiently by adopting the following **three** steps sequentially, namely :

Step 1 : Benzylpenicillin is first converted to the corresponding penicilloic acid (a dicarboxylic acid) by carrying out the hydrolysis with sodium hydroxide solution, as follows :



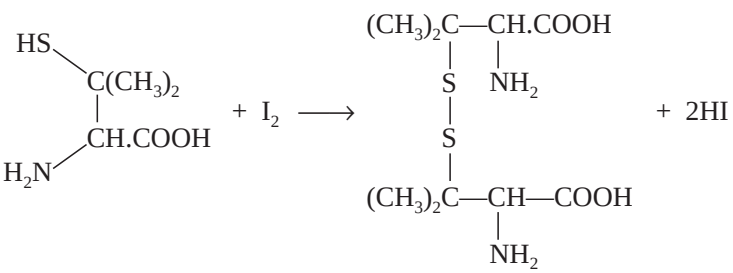
Benzylpenicillin — Penicilloic acid

Step 2 : Penicilloic acid on treatment with acid yields D-penicillamine and benzylpenilic acid, as shown under :



Benzylpenicillic acid — D-penicillamine

Step 3 : D-Penicillamine thus obtained is oxidised quantitatively by iodine to give rise to a disulphide, as expressed in the following equation ; whereas, the excess iodine is back titrated with 0.02 N sodium thiosulphate solution :



From the above reaction, we have :

$$C_{16}H_{17}N_2NaO_4S \equiv I \equiv e$$

In usual practice, however, benzylpenicillin sodium is standardised against a chemical reference substance of pre-determined potency.

Materials Required : Benzylpenicillin : 0.1 g ; (N) sodium hydroxide solution : 5 ml ; buffer solution (5.44% w/v of CH_3COONa and 2.40% w/v of glacial acetic acid) : 20 ml ; (N) hydrochloric acid : 5 ml ; 0.02 N iodine solution : 25 ml ; 0.02 N sodium thiosulphate solution ; starch solution.

Procedure : Weigh accurately about 0.1 g of benzylpenicillin in DW and dilute to 100 ml in a volumetric flask. Transfer 10.0 ml to an iodine flask, add 5 ml of N sodium hydroxide and allow to stand for 20 minutes. Now, add 20 ml of freshly prepared buffer solution, 5 ml of N HCl and 25.0 ml of 0.02 N iodine solution. Close the flask with a wet glass-stopper and allow to stand for 20 minutes in a dark place (*i.e.*, protected from light). Titrate the excess of iodine with 0.02 N sodium thiosulphate, employing freshly prepared starch solution as an indicator added towards the end-point.

To another 10.0 ml of the initial solution add 20 ml of the buffer solution, allow to stand for 20 minutes in the dark and titrate with 0.02 N sodium thiosulphate, using starch solution, added towards the end of the titration as indicator.

The difference between the two titrations represents the volume of 0.02 N iodine equivalent to the total penicillins present in the given sample of benzylpenicillin. An assay may be carried out simultaneously by benzylpenicillin sodium (reference sample) so as to determine the exact equivalent of each ml of 0.02 N iodine.

Calculations : Calculate the potency in Units of penicillin from the declared number of Units of pencillin in benzylpenicillin sodium (reference sample).

7.3.1.2.2. *Sodium Metabisulphite*

Theory : Sodium metabisulphite in acidic medium (HCl) yields SO_2 which reacts with water to produce sulphurous acid. The generated sulphurous acid is quantitatively oxidized by iodine to sulphuric acid, and the excess iodine is subsequently back titrated with sodium thiosulphate. The various reactions can be expressed as shown below :

$$Na_2S_2O_5 + 2HCl \longrightarrow 2NaCl + H_2O + 2SO_2$$

or $$2\,[SO_2 + H_2O \longrightarrow H_2SO_3]$$

or $$2\,[SO_3^{2-} + H_2O \longrightarrow SO_4^{2-}\,2H^+ + 2e]$$

or $$2\,[I_2 + 2e \longrightarrow 2I^-]$$

or $$Na_2S_2O_5 \equiv 2I_2 \equiv 4e$$

or $$190.10 \text{ g } Na_2S_2O_5 \equiv 2I_2 \equiv 4000 \text{ ml N}$$

or $$47.525 \text{ g } Na_2S_2O_5 \equiv 1000 \text{ ml N}$$

or $$0.004753 \text{ g } Na_2S_2O_5 \equiv 1 \text{ ml of 0.1 N Iodine}$$

Materials Required : Sodium metabisulphite : 0.2 g ; 0.1 N Iodine solution ; hydrochloric acid ($\simeq$ 11.5 N) : 1 ml ; 0.1 N sodium thiosulphate ; starch solution.

Procedure : Weigh accurately about 0.2 g of sodium metabisulphite and dissolve in 50.0 ml of 0.1 N iodine solution and add 1 ml hydrochloric acid. Titrate the excess of iodine with 0.1 N sodium thiosulphate employing freshly prepared starch solution, added towards the end of the titration, as indicator. Each ml of 0.1 N iodine is equivalent to 0.0047453 g of $Na_2S_2O_5$.

7.3.1.2.3. *Cognate Assays*

A few other pharmaceutical substances may also be assayed by adopting the residual titration method as shown in Table 7.2.

Table 7.2 : Substances Assayed by Residual Titration of Excess Iodine with Sodium Thiosulphate

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Polyvinyl pyrrolidone	4.0 g	Starch solution	Each ml of 0.1 N iodine ≡ 0.005557 g of C_6H_9NO
2.	Mechlorethamine hydrochloride	0.10 g	-do-	Each ml of 0.1 N Na_2SO_5 ≡ 9.626 g of $C_5H_{11}Cl_2N$. HCl

7.3.2. IODOMETRIC ASSAYS

In iodometric determinations the pharmaceutical substance oxidizes KI in an acidic medium to produce an equivalent quantity of iodine that may be assayed by titration with a standard solution of sodium thiosulphate.

7.3.2.1. Chlorinated Lime

Chlorinated lime or bleaching powder, $CaOCl_2$, contains about 30% w/w of available chlorine.

Theory : Chlorinated lime reacts with acetic acid to produce a mole each of calcium acetate, hydrochloric acid and hydrochlorous acid. The two acids interact to give water and chlorine, and the latter reacts with HI to liberate iodine that can be estimated by titrating with 0.1 N sodium thiosulphate solution. The various reactions involved may be expressed as given below :

$$CaCl(OCl) + 2CH_3COOH \longrightarrow Ca(CH_3COO)_2 + HCl + HClO$$

$$HCl + HClO \longrightarrow H_2O + Cl_2$$

$$2HI + Cl_2 \longrightarrow 2HCl + I_2$$

or 35.46 g Cl ≡ 1000 ml N

or 0.003546 g ≡ 1 ml of 0.1 N Sodium Thiosulphate

Materials Required : Chlorinated lime : 4 g ; dilute acetic acid : 5 ml ; potassium iodide : 3 g ; acetic acid : 5 ml ; 0.1 N sodium thiosulphate solution.

Procedure : Weigh accurately 4.0 g of chlorinated lime and triturate it in a glass-pestle-mortar with a little DW. Transfer the paste quantitatively into a 1 litre volumetric flask and shake thoroughly. Take a 100 ml volumetric flask, rinse it with a small quantity of the suspension from the 1 litre flask and finally fill it up with the suspension. Rinse out a 250 ml iodine flask containing a little dilute acetic acid and a little of the suspension from the 1-litre flask in order to oxidise any inorganic substance present in the iodine flask. Finally, wash it thoroughly with DW. Now, transfer 100 ml of the suspension completely from the 100 ml volumetric flask to the iodine flask by washing the former repeatedly with DW. Add to it acetic acid 5 ml followed by KI 3.0 g and shake the contents of the flask thoroughly. Titrate the liberated iodine with 0.1 N sodium thiosulphate which is equivalent to 0.003546 g of chlorine.

From this value the percentage of chlorine present in the given sample of chlorinated lime can be calculated.

7.3.2.2. Ferric Ammonium Citrate

Theory : In ferric ammonium citrate it is taken for granted that the entire iron is oxidized to the Fe^{2+} state and practically little Fe^{2+} is present. Thus, the ferric ion present in a known amount of the sample liberates an equivalent amount of iodine from an acidified KI solution. Thus, we have :

$$2[Fe^{3+} + e \longrightarrow Fe^{2+}]$$

or $$2I^- \longrightarrow I_2 + 2e$$

or $2Fe \equiv 2Fe^{3+} \equiv 2e$

or 2×55.85 g Fe $\equiv$ 2000 ml N

or 55.85 g Fe $\equiv$ 1000 ml N

or 0.05585 g Fe $\equiv$ 1 ml of 0.1 N Sodium Thiosulphate

Materials Required : Ferric ammonium citrate : 0.5 g ; sulphuric acid conc. : 1 ml ; 0.1 N $KMnO_4$ solution : 50 ml ; hydrochloric acid : 15 ml ; potassium iodide : 2.0 g ; 0.1 N sodium thiosulphate.

Procedure : Weigh accurately about 0.5 g of ferric ammonium citrate and dissolve the sample in 15 ml DW. Add to it slowly 1 ml of sulphuric acid and warm gently to attain a yellow colouration so as to decompose the iron and ammonium citrate complex completely. Cool and add 0.1 N potassium permanganate solution dropwise from a burette to obtain a pink colour that persists for 5 seconds. To the resulting solution add hydrochloric acid 15 ml and potassium iodide 2.0 g, shake well and set aside for 3 minutes so that iodine may be liberated completely. Now, add 60 ml of water and titrate with 0.1 N sodium thiosulphate solution while shaking the contents continuously till a colourless end-point is achieved.

Precautions :

(*i*) Addition of excess of $KMnO_4$ solution must be avoided, since pink colour developed shall disappear within a short span, which may ultimately give false high results,

(*ii*) Washing down during the course of titration must be checked rigidly in order to maintain the right proportion of various substances in the solution,

(*iii*) End-point is almost colourless, hence starch indicator can be skipped totally, and

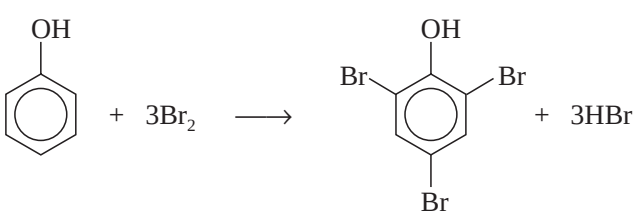
(*iv*) $KMnO_4$ oxidizes the traces of Fe^{2+} to Fe^{3+} in the sample, if any.

7.3.2.3. Thyroid

Thyroxine and diidotyrosine are the two iodine-substituted organic compounds which essentially constitute the active principles present in dried thyroid gland. The latter on being subjected to pyrolysis with anhydrous K_2CO_3, gives rise to an equivalent amount of KI present in the sample. Soon after the completion of carbonization, the crucible is cooled and the residue is extracted with water to dissolve KI, carbonates and other soluble compounds. The resulting solution is filtered and treated with Br_2 in the presence of phosphoric acid (H_3PO_4) so that complete oxidation of iodide to iodate is caused. The following reaction takes place :

$$I^- + 3Br_2 + 3H_2O \longrightarrow IO_3^- + 6HBr$$

The excess of bromine is removed by warming the acidic solution gently till the vapours show a negative test with starch-iodide paper. However, the residual traces of Br_2 are reduced by treatment of the resulting solution with phenol to yield the corresponding 2,4,6-tribromophenol as shown below :



Lastly, iodate (IO_3^-) in a weak acidic medium quantitatively oxidizes KI to an equivalent amount of iodine, as expressed below :

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

It is evident from the above equation that each gram-atomic weight of iodine in thyroid is converted to 1 mol of iodate and finally to 3 mol or 6 equivalent of iodine. Therefore, the equivalent weight of the iodine present in the dried thyroid gland is 21.15 g (*i.e.*, 1/6 × 127 At. wt. of I_2). Hence, each millilitre of 0.01 N sodium thiosulphate is equivalent to 0.0002115 g of iodine (*i.e.*, 0.01 × 0.02115 g).

Materials Required : Thyroid gland dried 1.0 g ; anhydrous potassium carbonate : 17.0 g ; bromine solution (9.6 ml of Br_2 and 30 g of KBr in 100 ml DW) : 7.0 ml ; dilute phosphoric acid (10% w/v) : 42.0 ml ; starch iodide paper ; phenol solution (saturated solution of phenol in water) : 5.0 ml ; potassium iodide solution (10% w/v in water) ; 0.01 N sodium thiosulphate solution ; starch solution.

Procedure : Weigh accurately about 1.0 g of dried thyroid gland in a porcelain crucible, add 7.0 g of anhydrous K_2CO_3, mix thoroughly and overlay with further 10 g more of anhydrous K_2CO_3, finally compact the mixture by tapping gently. Incenerate for 25 minutes at 675°—700°C in a preheated muffle furnace. Cool the contents, add 20 ml of DW, boil gently and decant through a filter paper into a flask. Repeat the extraction by boiling with 20 ml DW, wash the crucible and the residue on the filter with hot water until the filtrate is about 200 ml. To it add 7.0 ml of freshly prepared bromine solution followed by 40 ml of dilute phosphoric acid and continue boiling slowly till starch iodide paper is no longer coloured blue by the vapours. While boiling is in progress top up the volume to 200 ml by adding DW at intervals. Cool and add 5 ml of phenol solution and allow to stand for 5 minutes. Add 2 ml of dilute phosphoric acid and 5 ml of potassium iodide solution and titrate immediately with 0.01 N sodium thiosulphate solution employing starch solution as indicator towards the end-point. A blank estimation is also carried out simultaneously and necessary correction incorporated. Each ml 0.1 N sodium thiosulphate is equivalent to 0.0002115 g of I.

Precautions :

(*i*) Potassium carbonate should be perfectly anhydrous otherwise decrepitation would take place causing loss of material during pyrolysis,

(*ii*) Both the temperature of the muffle furnace and the extent of heating should be monitored closely, because KI is significantly volatile at an elevated temperature and part of it may be lost due to extended heating, and

(*iii*) The solution from which excess Br_2 is removed by heating must be acidic, otherwise a portion of Br_2 shall be fixed in the form of potassium hypobromite (KBrO).

7.3.2.4. Cognate Assays

A few pharmaceutical substances can be assayed by titrating the liberated iodine from potassium iodide with sodium thiosulphate as stated in Table 7.3.

Table 7.3 : Substances Assayed by Titrating the Liberated Iodine from Potassium Iodide with Sodium Thiosulphate

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Diiodohydroxy quinoline	12 mg	Starch solution	Each ml of 0.02 N $Na_2S_2O_3$ ≡ 0.6616 mg of $C_9H_5I_2NO$
2.	Mannitol	0.4 g	-do-	Each ml of 0.1 N I_2 ≡ 0.001822 g of $C_6H_{14}O_6$
3.	Phenindione	0.3 g	-do-	Each ml of 0.1 N $Na_2S_2O_3$ ≡ 0.01111 g of $C_{15}H_{10}O_2$

THEORETICAL AND PRACTICAL EXERCISES

1. What is the basic difference between '**iodimetric**' and '**iodometric**' titrations ? Explain with the help of equations involved in such typical titrations.

2. Iodimetic titrations may be accomplished by *two* methods :

(*a*) Direct titration with iodine

(*b*) Residual titration method.

Explain the above with the help of preparation of 0.1 N I_2-solution, its standardization and methodologies adopted.

3. How would you assay the following '**drugs**' by iodimetric titrations :
(*i*) Analgin (*ii*) Ascorbic Acid
(*iii*) Benzylpenicillin (*iv*) Mechlorethamine Hydrochloride.

4. Explain the assay of the following '**drug substances**' by using '**direct iodometic method**' :
(*i*) Chlorinated line (*ii*) Ferric Ammonium Citrate
(*iii*) Thyriod (*iv*) Mannitol.

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C. PRECIPITATION METHODS

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8 ARGENTOMETRIC METHODS

CONTAINS :

8.1 Introduction
8.2 Theory
8.3 Assay Methods
8.3.1 Direct titration with siliver nitrate
8.3.2 Ammonium thiocyanate—silver nitrate titrations

8.1. INTRODUCTION

In general, titrations governed by precipitation reactions do not really constitute an appreciable number in volumetric determinations in comparison to either redox or acid-base reactions. The interaction between silver-nitrate and sodium chloride in solutions result into the precipitation of silver chloride as shown below :

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

In actual practice, however, such titrations are more or less restricted to those involving precipitation of Ag^+ with anions, for instance : halogens (Cl^-, Br^-, I^-) and thiocyanate (SCN^-). Generally, it is quite difficult and tedious to locate the exact point at which further addition of reagent affords no more precipitation. Therefore, the choice and wisdom of a chemical reaction is preferably sought so as to result in either a coloured solution or a coloured precipitate at the end point. A typical instance may be cited by application of potassium chromate (K_2CrO_4) solution in the above case whereby any extra drop of silver nitrate, after all the chloride has been precipitated, immediately causes precipitation of red chromate showing that the end point has been duly achieved.

It is, however, interesting to observe here that such reactions do offer limited usage because of the following *two* facts, namely :

(*i*) Co-precipitation effects do not give a real composition of the precipitate, and

(*ii*) Choice of appropriate indicator is very much limited.

Besides, the foregoing facts another vital aspect to be taken into consideration is the **solubility** product that plays a major role in such titration. Hence, the equilibrium constant of the reaction giving the precipitate of AgCl may be expressed as :

$$AgCl\ (s) \longrightarrow Ag^+\ (aq) + Cl^-\ (aq)$$

or
$$K = \frac{[Ag^+][Cl^-]}{[AgCl]}$$

From the above expression the solubility product constant K*sp* may be designated as :

$$K_{sp} = [Ag^+]\ [Cl^-]$$

assuming the activity of solid AgCl being constant.

Following are the *four* cardinal parameters that may be considered for a feasible argentometric analysis, namely :

(*i*) Precipitate formed must be insoluble,

(*ii*) Precipitation process should be fast and rapid,

(*iii*) Co-precipitation effects must be minimal, and

(*iv*) Detection of equivalence point must be apparently visible.

8.2. THEORY

In the precipitation reaction involving chloride and silver nitrate, the addition of even a small quantity of the latter shall effect precipitation of AgCl provided that K*sp* has been exceeded significantly. At this juncture, the concentrations of both Ag^+ and Cl^- are related by the solubility-product equilibrium constant ; thus, we have :

$$Ag^+ \text{ (titrant)} + Cl^- \text{ (analyte)} \rightleftharpoons AgCl(s)$$

Chromate ion concentration required to initiate the precipitation of Ag_2CrO_4 commences at the equivalence point and may be calculated with the solubility products for AgCl and Ag_2CrO_4 :

$$AgCl : Ksp = 1.8 \times 10^{-10} = [Ag^+]\,(Cl^-)$$

$$Ag_2CrO_4 : Ksp = 1.2 \times 10^{-12} = [Ag^+]^2\,[CrO_4^{2-}]$$

Assuming that at the equivalence point,

$$[Ag^+] = 1.3 \times 10^{-5} \text{ M}$$

the chromate ion concentration must be :

$$[CrO_4^{2-}] = \frac{[AgCrO_4]}{[Ag^+]^2}$$

or

$$= \frac{1.2 \times 10^{-12}}{[1.3 \times 10^{-5}]^2}$$

or

$$= 6.7 \times 10^{-3} \text{ M}$$

In actual practice, the concentration of chromate produces an intense yellow colour to such an extent that the end point is masked. Therefore, normally concentrations of 5×10^{-3} M are employed in analytical procedures. It suggests that $[Ag^+]$ shall be $> 1.3 \times 10^{-5}$ M at the end-point thereby introducing a positive determinate error. However, it has been proved experimentally that even with concentrations as low as 2×10^{-3} M, the extent of error caused is negligibly small.

Adsorption-coprecipitation phenomenon using fluorescein, dichlorofluorescein and tetrabromofluorescein (eosin) essentially impart the fluoresceinate ion that is absorbed on the AgCl particles. At the equivalence point, the AgCl particles change from white to pink due to the coprecipitation of silver fluoresceinate. In short, the adsorption indicator method is quite rapid and capable of providing very accurate results for the estimation of Cl^- with $AgNO_3$.

Furthermore, Br^-, I^- and SCN^- ions can also be titrated with $AgNO_3$ employing eosin as an adsorption indicator.

8.3. ASSAY METHODS

Argentometric titrations may be divided into *two* broad categories, namely :

(*i*) Direct titration with silver-nitrate, and

(*ii*) Ammonium thiocyanate-silver nitrate titrations (Volhard's Method).

8.3.1. DIRECT TITRATION WITH SILVER NITRATE

Pharmaceutical substances essentially containing halides may be estimated by direct titration with silver nitrate solution as a titrant.

8.3.1.1. Preparation of 0.1 N Silver Nitrate Solution

Materials Required : Silver nitrate (AR) : 16.989 g.

Procedure : Weigh accurately 16.989 g of silver nitrate on a watch-glass and transfer quantitatively into a 1 litre volumetric flask. Add freshly prepared DW and make up the volume to 1000 ml. Thus, we have :

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

or $AgNO_3 \equiv NaCl \equiv H$

or $169.89 \text{ g } AgNO_3 \equiv 58.45 \text{ g NaCl} \equiv 1000 \text{ ml N}$

or $0.01699 \text{ g } AgNO_3 \equiv 0.005845 \text{ g NaCl} \equiv 1 \text{ ml } 0.1 \text{ N } AgNO_3$

8.3.1.2. Standardization of 0.1 N Silver Nitrate Solution

Materials Required : Sodium chloride : 0.1 g ; acetic acid (33% w/v) : 5 ml ; methyl alcohol (95%) : 50 ml ; eosin solution (0.5% w/v in water) : 5 ml ; 0.1 N silver nitrate solution.

Procedure : Weigh accurately about 0.1 g of sodium chloride, previously dried at 110°C for 2 hours, and dissolve in 5 ml of water. Add 5 ml of acetic acid, 50 ml of methyl alcohol and three drops of eosin solution. Stir thoroughly on a magnetic stirrer and titrate with the silver nitrate solution till the white particles of AgCl change from white to pink. Each 0.005844 g of sodium chloride is equivalent to 1 ml of 0.1 N silver nitrate.

8.3.1.2.1. *Potassium Chlroride*

Materials Required : Potassium chloride : 0.25 g ; potassium chromate solution (5% w/v in water) : 10 ml ; 0.1 N silver nitrate solution.

Procedure : Weigh accurately about 0.25 g of potassium chloride in a conical flask and dissolve it in 50 ml of DW and titrate with 0.1 N silver nitrate solution, using 2-3 drops of potassium chromate solution as indicator till precipitation of red chromate is indicated. Each ml of 0.1 N silver nitrate solution is equivalent to 0.007455 g of KCl.

Equations :

$$AgNO_3 + KCl \longrightarrow AgCl \downarrow + KNO_3$$

or $AgNO_3 \equiv KCl \equiv H$

or $169.89 \text{ g } AgNO_3 \equiv 74.55 \text{ g KCl} \equiv 1000 \text{ ml N}$

or $0.01699 \text{ g } AgNO_3 \equiv 0.007455 \text{ g KCl} \equiv 1 \text{ ml of } 0.1 \text{ N } AgNO_3$

8.3.1.2.2. *Chloral Hydrate*

Materials Required : Chloral hydrate : 4.0 g ; sodium hydroxide (N) : 30 ml ; sulphuric acid (N) ; phenolphthalein solution (1.0% w/v in 50% v/v alcohol) ; 0.1 N silver nitrate solution ; potassium chromate solution (5% w/v in water).

Procedure : Weigh accurately about 4 g of chloral hydrate and dissolve in 10 ml of DW and add 30 ml of N sodium hydroxide solution. Allow the resulting mixture to stand for 2 minutes, and then titrate with N sulphuric acid, employing phenolphthalein solution as indicator till a colour change from pink to colourless is achieved. Titrate the neutralized liquid thus obtained with 0.1 N silver nitrate using potassium chromate solution as indicator till precipitation of red chromate is obtained, Add, now 2/15th of the amount of 0.1 N silver nitrate used to the amount of N sulphuric acid used in the first titration and deduct the figure so obtained

from the amount of N sodium hydroxide added. Each ml of N sodium hydroxide, obtained as difference, is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

Explanation :

(*i*) The estimation depends upon the interaction between chloral hydrate and sodium hydroxide as shown by the following equation :

$$C.Cl_3CH(OH)_2 + NaOH \longrightarrow CHCl_3 + HCOONa + H_2O \quad ...(a)$$

$$CCl_2CH(OH)_2 \equiv NaOH \equiv H$$

or $165.40 \text{ g } C_2H_3O_2Cl_3 \equiv 1000 \text{ ml N}$

or $0.1654 \text{ g } C_2H_3O_2Cl_3 \equiv 1 \text{ ml N NaOH}$

(*ii*) As the chloroform generated in Eq. (*a*) undergoes chemical reaction with the alkali to a certain degree; therefore, addition of alkali followed by back titration does not afford the correct assay. Thus, we have :

$$CHCl_3 \xrightarrow{\text{Base}} \underbrace{H.COOH + HCl}_{\downarrow 4NaOH}$$

$$H.COONa + 3NaCl + 4H_2O \quad ... (b)$$

Hence, from Eq. (*b*) we have :

$$CHCl_3 \equiv 4NaOH$$

(*iii*) The ionized chloride generated from the additional side reaction (*b*) may be estimated by titration with 0.1 N silver nitrate solution, and necessarily a correction has got to be made to the alkali-titration reading so as to adequately compensate for this side reaction. Thus, from equation (*b*) we have :

$$CHCl_3 \equiv 4NaOH \equiv 4000 \text{ ml N solution}$$

Also, $3NaCl \equiv 3000 \text{ ml of N } AgNO_3 \text{ solution}$

or $3NaCl \equiv 30{,}000 \text{ ml of 0.1 N } AgNO_3 \text{ solution}$

Therefore, it is evident that 2/15th of the volume of 0.1 N $AgNO_3$ (*i.e.*, 2/15th of 30,000 = 4,000) needed shall give the volume of N NaOH that reacted with chloroform as per Eq. (*b*).

8.3.1.2. Cognate Assays

The pharmaceutical substances in Table 8.1, can be assayed by direct titration with silver nitrate using a suitable indicator.

Table 8.1 : Substances Assayed by Direct Titration with Silver Nitrate

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Iopanoic acid	0.25 g	Tetrabromo-phenolphthalein ethyl ester	Each ml of 0.05 N $AgNO_3 \equiv 9.516$ mg of $C_{11}H_{12}I_3NO_2$
2.	Benzyltrimethyl ammonium chloride	2.0 ml	Dichloro-fluorescein	Each ml of 0.1 N $AgNO_3 \equiv 18.57$ mg of $C_6H_5CH_2N(CH_3)Cl$
3.	Diatrizoate sodium	0.30 g	Tetrabromo-phenolphthalein ethyl ester	Each ml of 0.05 N $AgNO_3 \equiv 10.60$ mg of $C_{11}H_8I_3N_2NaO_4$

8.3.2. AMMONIUM THIOCYANATE-SILVER NITRATE TITRATIONS (VOLHARD'S METHOD)

Volhard's method is based on *two* major aspects, namely :

(*a*) Complete precipitation of insoluble silver salts from nitric acid solution by adding an excess of silver nitrate solution to a corresponding soluble salt, and

(*b*) Estimation of excess of silver nitrate solution by carrying out residual titration with standard ammonium thiocyanate solution, employing ferric ammonium sulphate as an indicator.

Thus, ammonium thiocyanate reacts with silver nitrate in nitric acid solution as below :

$$NH_4SCN + AgNO_3 \longrightarrow AgSCN \downarrow + NH_4NO_3$$

However, in actual practice the thiocyanate solution is always taken in the burette and is run directly into the silver nitrate solution in the flask that has been duly acidified with nitric acid. Ferric ammonium sulphate is the choicest indicator since the end point is visibly detected by a deep red colour (ferric thiocyanate) due to the interaction of Fe^{2+} ions with a trace of SCN^- ion.

Precautions :

(*i*) Nitric acid must be free from nitrous acid, otherwise thiocyanic acid may give an instant red colouration, and

(*ii*) Temperature of the solution should be maintained below 25°C since at an elevated temperature the red colour of the ferric thiocyanate complex fades away rapidly. Therefore, we have :

$$NH_4SCN \equiv AgNO_3 \equiv H$$

8.3.2.1. Preparation of 0.1 N Ammonium Thiocyanate Solution

Materials Required : Ammonium thiocyanate : 8.0 g.

Procedure : Weigh about 8.0 g of ammonium thiocyanate and transfer it quantitatively in 1 litre volumetric flask. Dissolve it in DW and make up the volume upto the mark.

Equation :

$$NH_4SCN \equiv AgNO_3 \equiv H$$

or $76.12 \text{ g } NH_4SCN \equiv 1000 \text{ ml N}$

or $7.612 \text{ g } NH_4SCN \equiv 1000 \text{ ml } 0.1 \text{ N } AgNO_3$

8.3.2.2. Standardization of 0.1 N Ammonium Thiocyanate Solution

Materials Required : 0.1 N Silver nitrate solution : 25 ml ; nitric acid (16 N) : 2 ml ; ferric ammonium sulphate (10% w/v in water) : 2 ml ; 0.1 N ammonium thiocyanate solution.

Procedure : Pipette 25 ml of a standard 0.1 N $AgNO_3$ solution into a glass-stoppered flask (iodine-flask), dilute with 50 ml of DW, add to it 2 ml of nitric acid and 2 ml of ferric ammonium sulphate solution and titrate with ammonium solution to the first appearance of red-brown colour. Each ml of 0.1 N silver nitrate is equivalent to 0.007612 g of NH_4SCN.

Note : Soon after the addition of ammonium thiocyanate a white precipitate of silver thiocyanate is formed first and then a reddish-brown colour appears that fades out completely upon shaking thereby leaving a white precipitate of silver thiocyanate. The end-point is indicated by the appearance of a permanent faint reddish brown colour that does not vanish upon shaking.

8.3.2.2.1. *Chlorobutol*

Materials Required : Chlorobutol : 0.2 g ; alcohol (95%) : 5 ml ; sodium hydroxide solution (20% w/v in water) : 5 ml ; nitric acid (16 N) : 5 ml ; nitrobenzene : 1 ml ; 0.1 N silver nitrate solution : 50 ml ; ferric ammonium sulphate solution (10% w/v in water) ; 0.1 N ammonium thiocyanate solution.

Procedure : Weigh accurately about 0.2 g of chlorobutol in a flask and dissolve in 5 ml of alcohol. Add to it 5 ml of sodium hydroxide solution, and boil under a reflux condenser for 15 minutes. Cool, dilute with 20 ml of DW, add 5 ml of nitric acid, 1 ml of nitrobenzene and 50 ml of 0.1 N silver nitrate solution. Shake the contents vigorously for 1 minute, add 4 ml of ferric ammonium sulphate solution and titrate the excess of silver nitrate with 0.1 N ammonium thiocyanate solution. Each ml of 0.1 N silver nitrate is equivalent to 0.005917 g of $C_4H_7Cl_3O$.

Explanation : Chlorine combined originally to chlorobutol is being converted by hydrolysis in the presence of sodium hydroxide to ionic chloride that may be estimated quantitatively by Volhard's method in the presence of nitrobenzene.

Thus, we have :

$$CCl_3C(CH_3)_2.OH + 3NaOH \longrightarrow C(OH)_3.C(CH_3)_2.OH + 3NaCl$$

$$\downarrow \text{NaOH}$$

$$(CH_3)_2.C(OH).COONa$$

or $$3NaCl + 3AgNO_3 \longrightarrow 3NaNO + 3AgCl \downarrow$$

or $$C_4H_7OCl_3 \equiv 3NaCl \equiv 3AgNO_3 = 3H \equiv 3000 \text{ ml N}$$

or 186.48 g of $C_4H_7OCl_3 \equiv 3000$ ml N

or 0.006216 g $C_4H_7OCl_3 \equiv 1$ ml of 0.1 N $AgNO_3$

8.3.2.2.2. *Ethionamide*

Theory : Theoretically the cleavage of thioamide link in ethionamide takes place in an acidic medium. Subsequent neutralization with NH_4OH yields ammonium sulphide which on addition of silver nitrate yields a precipitate of Ag_2S. Thus we have :

$$C_8H_{10}N_2S \equiv CS_2$$

or $$CH_2 + NH_4OH \longrightarrow 2(NH_4)_2S + 2H_2O + CO_2$$

or $$(NH_4)_2S + 2AgNO_3 \longrightarrow Ag_2S \downarrow + 2NH_4NO_3$$

Materials Required : Ethionamide : 0.3 g ; dilute sulphuric acid (10% w/w) : 10 ml ; dilute ammonia solution (4.25 ml of strong ammonia solution in 100 ml of water) ; 0.1 N silver nitrate : 50 ml ; dilute nitric acid (10.6 ml of nitric acid to 100 ml of water) : 60 ml ; ferric ammonium sulphate solution (10% w/v in water) : 5 ml ; and 0.1 N ammonium thiocyanate solution.

Procedure : Weigh accurately about 0.3 g of ethionamide in a flask and dissolve in 10 ml of dilute sulphuric acid. Add to it 100 ml of water, 20 ml of dilute ammonia solution and rapidly 50 ml of 0.1 N silver nitrate solution. Allow the resulting mixture to stand for a few minutes, filter and wash the filter paper with three successive quantities, each of 10 ml of DW. To the combined filtrate and washings, add 60 ml of dilute nitric acid, cool and titrate with 0.1 N ammonium thiocyanate employing 5 ml of ferric ammonium sulphate solution as an indicator. Each ml of 0.1 N silver nitrate is equivalent to 0.008312 g of $C_8H_{10}N_2S$.

8.3.2.2.3. *Cognate Assays*

A good number of pharmaceutical substances can be assayed by Volhard's method and are mentioned in Table 8.2.

Table 8.2 : Substances Assayed by Volhard's Method

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Aminophylline	0.25 g	Ferric ammonium sulphate	Each ml of 0.1 N $AgNO_3 \equiv 0.02102$ g of $C_{16}H_{24}N_{10}O_4$
2.	Chlorophenothane	1.0 g	-do-	Each ml of 0.1 N $AgNO_3 \equiv 0.00709$ g of $C_{14}H_9Cl_5$
3.	Dimenhydrinate (for 8-chloro-theophylline)	0.8 g	-do-	Each ml of 0.1 N $AgNO_3 \equiv 0.02146$ g of $C_7H_7ClN_4O_2$
4.	Gamma Benzene Hexachloride*	0.4 g	-do-	Each ml of 0.1 N $AgNO_3 \equiv 0.009694$ g of $C_6H_6Cl_6$
5.	Oxyphenonium Bromide	0.8 g	-do-	Each ml of 0.1 N $AgNO_3 \equiv 0.04284$ g of $C_{21}H_{34}BrNO_3$
6.	Sodium Chloride	0.1 g	-do-	Each ml of 0.1 N $AgNO_3 \equiv 0.005844$ g of NaCl

* Hydrolysis with ethanolic KOH helps in the conversion of organically combined chlorine to KCl which after due acidification with HNO_3 is assayed by Volhard's Method.

THEORETICAL AND PRACTICAL EXERCISES

1. Explain the following :
(*i*) Precipitation reactions governing '**argentometric methods**'.
(*ii*) Role of '**solubility product**' in precipitation reactions.
(*iii*) Various cardinal parameters required for a feasible argentometric analysis.

2. Discuss the '**theoretical aspect**' of argentometric methods explicitely.

3. Give a comprehensive account of the '**direct titration method**' with silver nitrate with reference to the following :
(*a*) Preparation 0.1 N $AgNO_3$ solution (1 L)
(*b*) Standardization of 0.1 N $AgNO_3$ solution
(*c*) Assay of chloral hydrate.
(*d*) Assay of Benzyltrimethyl ammonium chloride.

4. (*a*) What is Volhard's method ? Explain it with the help of equations and the precautions involved in it.
(*b*) Preparation and standardization of 0.1 N Ammonium Thiocyanate solution.
(*c*) Describe the assay of the following '**drugs**',
(*i*) Chlorobutol (*ii*) Ethionamide
(*iii*) Aminophylline (*iv*) Dimenhydrinate.

RECOMMENDED READINGS

1. Gordon, M., L. Salutsky and H.H. Willard, '**Precipitation from Homogeneous Solution**', New York, John Wiley & Sons, 1959.

2. Kolthoff, I.M., P.J. Elving, and E.B. Sandell, eds., '**Treatise on Analytical Chemistry**', Pt. 1, Vol, 1, New York, Interscience Publishers, 1959.

3. Ayers, C., '**Argentometric Methads**', In **Comprehensive Analytical Chemistry**', ed. by Wilson, C.L., and D.W. Wilson, Vol. 1B., New York, Elsevier North-Holland, 1960.

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D. COMPLEXOMETRIC METHODS

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9 COMPLEXOMETRIC ANALYSIS

CONTAINS :

9.1 Introduction
9.2 Theory
- 9.2.1 Effect of pH on complexation
- 9.2.2 Stability of complexes
- 9.2.3 Colouration of complexes
- 9.2.4 Titrability of polyvalent metal ions employing disodium acetate
- 9.2.5 Usage of pM indicators in complexometric titrations

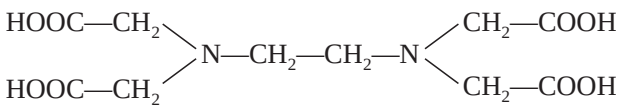
9.3 Assay methods
- 9.3.1 Direct titration methods
- 9.3.2 Masking and demasking agents
- 9.3.3 Residual titration methods

9.1. INTRODUCTION

A host of inorganic pharmaceutical substances essentially containing polyvalent and bivalent metal ions, for instance : Al^{3+}, Ca^{2+}, Mg^{2+}, Bi^{2+} and Zn^{2+} were initially analysed quantitatively by the aid of *gravimetric methods*. These historically important procedures have become more or less obsolete since they are extremely time consuming and tedious owing to several steps involved *e.g.*, precipitation, filtration, washing, drying and finally ignition to a constant weight.

Thereafter, analysis was done by faster techniques based on oxalate-permanganate titrations which involved a precipitation procedure followed by quick titration of pre-heated solutions containing oxalate ions.

More recently the introduction of an analytical reagent disodium ethylene-diaminetetraacetate, invariably termed as **EDTA**, an altogether latest titrimetric method has been used exclusively for the estimation of metals using *metal-ion indicators*.



EDTA

9.2. THEORY

Complex is a compound that is formed by the combination of a metal ion with a molecule that is capable of donating electrons, for example :

$[Cu(NH_3)_4]^{2-}$ and $Co[(NH_3)_6]^{3+}$

Cupric ammonium ion Cobaltammine

In the above two examples both Cu^{2+} and Co^{2+} form complexes with lone pair of electrons present in the neutral molecule ammonia *e.g.*, $\ddot{N}H_3$.

Chelate is a complex that is formed by the combination of a polyvalent metal ion with a molecule which essentially contains two or more groups that can donate electrons.

Specifically, disodium ethylenediaminetetraacetate (EDTA) reacts with **polyvalent metal ions** to result in the formation of a fairly stable *water-soluble complex*, or a *chelate compound*.

It is, however, pertinent to mention here that the predominant state of the dissociated forms of EDTA (*viz* . Y^{4-}, HY^{3-} , H_2Y^{2-} and H_3Y^{-}) is solely dependent upon the pH of the medium at which complexation takes place :

where, H_4Y = ethylenediaminetetraacetic acid, and

Y^{4-} = tetracetate ion.

In general, all EDTA complexation reactions essentially have the ratio of EDTA to metal ion as 1 : 1. Thus, we have :

$$Y^{4-} + M^{n+} \rightleftharpoons MY^{n-4}$$

Ligand is a molecule that affords groups for attachment to metal ions such as EDTA.

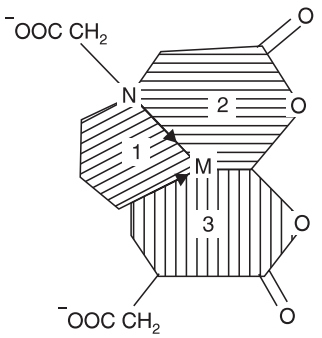
Some examples of polyvalent metal ions are given below :

Bivalent Metal ions : Ca^{2+}, Mg^{2+}, Zn^{2+},

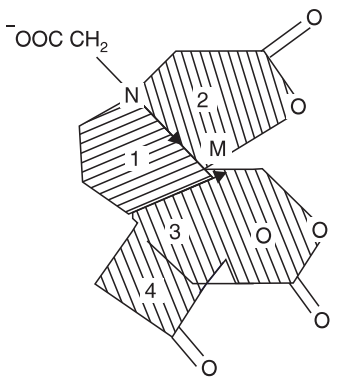
Trivalent Metal ions : Fe^{3+}, Al^{3+}, Cr^{3+},

Tetravalent Metal ions : Sn^{4+}, Ce^{4+}, Cr^{4+}, Pt^{4+}.

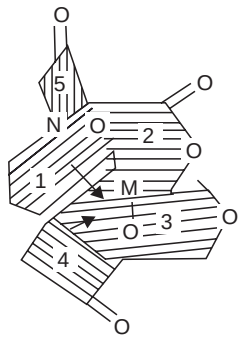
The structures of the complexes formed with *di-*, *tri-* and *tetra*-valent metal ions give rise to *three, four* and *five* rings respectively as depicted below :



M^{2+} [Ca^{2+}, Mg^{2+}, Zn^{2+}]
Nos. of rings formed = 3



M^{3+} [Fe^{3+}, Al^{3+}, Cr^{3+}]
Nos. of rings formed = 4



M^{4+} [Sn^{4+}, Ce^{4+}, Cr^{4+}, Pt^{4+}]
Nos. of rings formed = 5

There are various aspects in complex formation and detection, namely :

(*i*) Effect of pH on complexation,

(*ii*) Stability of complexes,

(*iii*) Colouration of complexes,

(*iv*) Titrability of polyvalent metal ions employing disodium edetate, and

(*v*) Usage of pM indicators in complexometric titrations.

9.2.1. EFFECT OF pH ON COMPLEXATION

Ethylenediamine tetracetic acid (H_4Y) undergoes ionization at *four*, different stages, namely :

$[H^+ + H_3Y^- ; pK_1 = 2.07]$; $[H^+ + H_2Y^{2-} ; pK_2 = 2.75]$;

$[H^+ + HY^{3-} ; pK_3 = 6.24]$; $[H^+ + Y^{4-} ; pK_4 = 10.34]$.

In reality, the actual complexing species is the tetracetate ion *i.e.*, Y^{4-} ; therefore, complexation will take effect more efficiently and be more stable in an aikaline medium. Hence, it is evident that EDTA complexes of many divalent metals are quite stable in ammoniacal solution.

As we have seen earlier that the trivalent metal complexes are normally bound still more firmly due to the formation of four rings (unlike three rings with divalent metal complexes) and stable in strongly acidic solutions, for instance : cobalt (Co^{2+}) EDTA complex is fairly stable in concentrated hydrochloric acid ($\simeq$ 11.5 N).

Though a good number of metal-EDTA complexes are found to be quite stable over a wide-spectrum of pH, yet in actual practice solutions are normally buffered for *two* specific reasons :

(*a*) to stabilize the complex formed, and

(*b*) to achieve the most distinct colour-change of the indicator.

9.2.2. STABILITY OF COMPLEXES

Generally, the formation of a 1 : 1 chelate complex (MX) may be designated by the following equation :

$$M + X \rightleftharpoons MX$$

where, M = Metal ion, and

X = Chelating ion.

Hence, the stability constant, K, may be expressed as :

$$K = \frac{[MX]}{[M][X]}$$

where, items within the 'square brackets' represent activities.

There are *two* cardinal factors which influence the stability constant (K), namely :

(*a*) Elevation in temperature affords a slight enhancement in the ionization of the complex and a slight lowering of K, and

(*b*) Stability constant is decreased on the addition of electrolytes with no common ion ; whereas, ethyl alcohol enhances K, perhaps on account of the suppression of ionization.

Table 9.1, provides the values of the logarithms of stability constants (K) of EDTA-complexes of certain metals normally occurring in pharmaceutical substances :

Table 9.1 : Stability Constants of EDTA-Complexes

S.No.	Cation	Log K	Complexed Metal Ions
l.	Ba^{2+}	7.8	BaY^{2-}
2.	Mg^{2+}	8.7	MgY^{2-}
3.	Ca^{2+}	10.6	CaY^{2-}
4.	Zn^{2+}	16.5	ZnY^{2-}
5.	Cr^{3+}	24.0	CrY^{1-}
6.	Fe^{3+}	25.1	FeY^{1-}
7.	Al^{3+}	15.5	AlY^{1-}

9.2.3. COLOURATION OF COMPLEXES

The formation of EDTA-metal ion complexes invariably attribute a change in the absorption spectrum pattern which ultimately forms the basis of a large number of colorimetric assays.

9.2.4. TITRABILITY OF POLYVALENT METAL IONS EMPLOYING DISODIUM EDETATE

Ethylenediamine tetracetic acid is found to be sparingly soluble in water ($\simeq$ 0.2% w/v) whereas its corresponding disodium salt is almost 50 times more soluble than the parent compound (solubility $\simeq$ 10% w/v). Therefore, it is the disodium salt of EDTA which is normally used in complexometric titrations.

In actual practice, whenever the disodium EDTA solution is added to a solution of a metal ion previously buffered to augment complexation, it has been observed that initially the rate of change of concentration of metal ion is rather slow, but interestingly it picks up quite rapidly as further addition of sodium-EDTA approaches one equivalent.

9.2.5. USAGE OF pM INDICATORS IN COMPLEXOMETRIC TITRATIONS

The equivalence point in complexometric titrations is invariably observed by the help of pM indicators. The relationship amongst pM, concentrations of ligand, chelate complex and stability constant may be established by the following equations :

Assuming K as the stability constant, we have :

$$K = \frac{[MX]}{[M][X]}$$

or $$[M] = \frac{[MX]}{[M][K]}$$

or $$\log [M] = \log \frac{[MX]}{[X]} - \log K$$

or $$p[M] = \log \frac{[X]}{[MX]} - pK \qquad \text{... } (a)$$

Now, considering Eq. (*a*), if a solution is made in such a manner that [X] = [MX], we have :

$$pM = - pK$$

or $$pM = pK' \quad \text{... (}b\text{)}$$

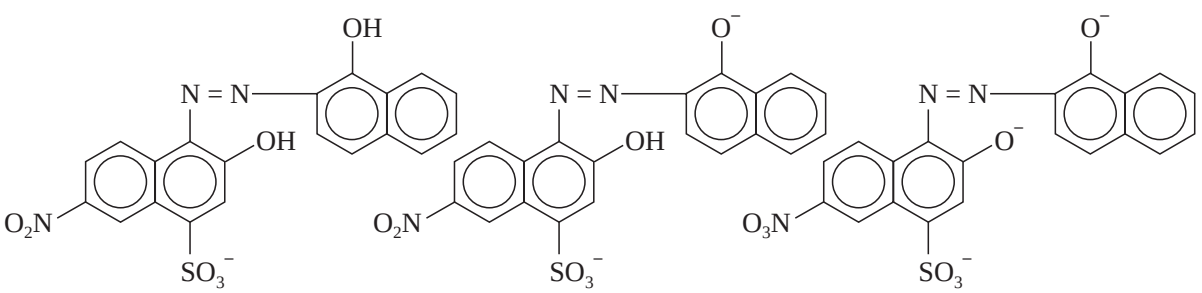
where, K′ is the dissociation constant.

From Eq. (*b*) it may be concluded that a solution having equal activities of free chelating agent and the metal-complex formed, the concentration of metal ions shall remain almost constant and would be buffered exactly in a similar fashion as are H^+ ions in a pH-buffer. As we know that the various chelating agents are mostly basic in character, therefore, the equilibrium attained in a metal-buffer solution is largely influenced by a change in pH. Hence, it may be concluded that the amino acid type chelating agents, such as : ethylenediamine tetracetic acid and ammoniatriacetic acid, when [X] = [MX], pM increases proportionately with pH until it reaches a value pH 10, thereby attaining a constant value. Hence, this particular pH is the '**Ideal pH**' at which complexometric titrations of metals with chelating agents in buffered solution must be performed.

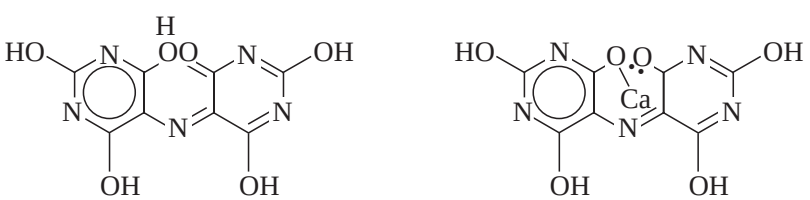
pM Indicator : It is a dye that serves as a chelating agent to yield a *dye-metal complex*, which apparently differs in colour from the original dye, besides possessing a lower stability constant than the corresponding *chelate-metal complex*. Hence, the colour imparted to the solution is mostly attributed due to the dye-complex formed until the end-point, when an equivalent amount of sodium-EDTA has been incorporated. The critical point at which the metal-dye complex decomposes to yield free-dye on addition of the slightest excess of sodium-EDTA, is distinctly shown by a visible change in colour.

Examples :

(*i*) **Mordant Black 2** : (Syn. : Eriochrome Black T ; Solochrome Black T)



(*ii*) **Murexide :** (Syn. : Ammonium Purpurate)



9.3. ASSAY METHODS

The complexometric titrations involving various inorganic pharmaceutical substances may be categorized into *three* broad heads, namely :

(*i*) Direct titration methods,

(*ii*) Masking and demasking agents, and

(*iii*) Residual titration methods.

9.3.1. DIRECT TITRATION METHODS

In direct titration, usually an appropriate buffer solution and a suitable indicator are added to the M^{2+} (metal-ion) solution and subsequently the resulting solution is titrated with previously standardized disodium-EDTA until the indicator just changes colour. Sometimes, a simultaneous blank determination is also recommended to have a check for the presence of traces of metallic impurities in the reagents.

9.3.1.1. Preparation of 0.05 M Disodium Ethylenediamine Tetracetate Solution (Disodium Edetate 0.05 M)

Materials Required : Disodium ethylenediaminetetracetate : 18.6 g.

Procedure : Weigh accurately 18.6 g of disodium ethylenediaminetetracetae, dissolve in sufficient DW in a 1 litre volumetric flask and make up the volume upto the mark.

Calculations :

$$\underset{(372.24)}{C_{l0}H_{14}N_2Na_2O_8,\ 2H_2O} \equiv Ca^{2+} \equiv 1000 \text{ ml M}$$

or 372.24 g $C_{10}H_{14}N_2Na_2O_8, 2H_2O \equiv 1000$ ml M

or 18.612 g $C_{10}H_{14}N_2Na_2O_8, 2H_2O \equiv 1000$ ml 0.05 M

or 0.01861 g $C_{10}H_{14}N_2Na_2O_8, 2H_2O \equiv 1$ ml of 0.05 M Disodium Edetate.

9.3.1.2. Standardization of 0.05 M Disodium Edetate Solution

Materials Required : Granulated zinc : 0.8 g ; dilute HCl (10% w/v of HCl) : 12.0 ml ; bromine water (3 ml Br_2 in 100 ml H_2O) : 5 ml ; sodium hydroxide (2 N) : 20.0 ml ; ammonia buffer (pH 10.0) (dissolve 5.4 g of NH_4Cl in 70 ml of 5 N ammonia and dilute with water to 100 ml) : 100 ml ; Mordant Black II mixture (mixture of 0.2 part Mordant Black II with 100 parts of NaCl) : 50 mg ; disodium edetate : 0.05 M.

Procedure : Weigh accurately about 0.8 g of granulated zinc, dissolve by gentle warming in 12 ml of dilute hydrochloric acid and 5 drops of bromine water. Boil to remove excess bromine, cool and add sufficient DW to produce 200 ml in a volumetric flask. Pipette 20 ml of the resulting solution into a flask and neutralize carefully with 2 N sodium hydroxide. Dilute to about 150 ml with DW, add to it sufficient ammonia buffer (pH 10.0) to dissolve the precipitate and add a further 5 ml quantity in excess. Finally add 50 mg of Mordant Black II mixture and titrate with the disodium edetate solution until the solution turns green. Each 0.003269 g of granulated zinc is equivalent to 1 ml of 0.05 M disodium ethylenediaminetetracetate.

Calculations :

$$ZnCl_2 \equiv Zn^{2+} \equiv 1000 \text{ ml M}$$

or 65.38 g Zn ≡ 1000 ml M

or 3.269 g Zn ≡ 1000 ml 0.05 M

or 0.003269 g Zn ≡ 1 ml of 0.05 M Disodium ethylenediaminetetracetate

9.3.1.3. Calcium Chloride

Materials Required : Calcium chloride dihydrate : 0.15 g ; dilute hydrochloric acid (10% w/w of HCl) : 3.0 ml ; 0.05 M disodium edetate ; sodium hydroxide solution (20% w/v in water) ; calcon mixture (a mixture of 1 part of calcon with 99 parts of freshly ignited anhydrous Na_2SO_4) : 0.1 g.

Equations :

$$Ca^{2+} + [H_2X]^{2-} \longrightarrow [CaX]^{2-} + 2H^+$$

or $CaCl_2.2H_2O \equiv Ca^{2+} \equiv Na_2H_2X, 2H_2O$

or 147.02 g $CaCl_2, 2H_2O \equiv$ 20,000 ml 0.05 M

or 0.007351 g $CaCl_2, 2H_2O \equiv$ 1 ml of 0.05 M Disodium Edetate

Procedure : Weigh accurately about 0.15 g of calcium chloride dihydrate and dissolve it in 50 ml of DW. Titrate with 0.05 M disodium ethylenediamine tetracetate to within a few ml of the expected end point, add 8.0 ml of sodium hydroxide solution and 0.1 g of calcon mixture and continue the titration until the colour of the solution changes from pink to a full blue colour. Each ml of 0.05 M disodium ethylene disodium tetracetate is equivalent to 0.007351 g of $CaCl_2 . 2H_2O$.

9.3.1.4. Magnesium Sulphate

Materials Required : Magnesium sulphate heptahydrate : 0.3 g ; strong ammonia-ammonium chloride solution (6.75 g NH_4Cl in 74.0 ml strong ammonia solution add water q.s. to produce to 100 ml) ; 0.05 M disodium edetate ; Mordant Black II mixture (mixture of 0.2 part mordant black II with 100 parts of NaCl) : 0.1 g.

Equations :

The assay of $MgSO_4.7H_2O$ is based upon the reactions designated by the following equations :

$$Mg^{2+} + [H_2X]^{2-} \longrightarrow [MgX]^{2-} + 2H^+$$

or $MgSO_4.7H_2O \equiv Mg^{2+} \equiv Na_2H_2X, 2H_2O$

or 120.38 g $MgSO_4 \equiv$ 20,000 ml 0.05 M

or 0.00602 g $MgSO_4 \equiv$ 1 ml of 0.05 M Disodium Edetate

Procedure : Weigh accurately about 0.3 g of magnesium sulphate heptahydrate and dissolve in 50 ml of DW. Add to it 10 ml of strong ammonia-ammonium chloride solution, and titrate with 0.05 M disodium ethylenediaminetetracetate employing 0.1 g of mordant black II mixture as indicator, until the pink colour is discharged from the blue. Each ml of 0.05 M disodium ethylenediaminetetracetate is equivalent to 0.00602 g of $MgSO_4$.

9.3.1.5. *Cognate Assays*

A number of pharmaceutical inorganic substances may be assayed by the direct titration method using disodium ethylenediaminetetracetate. A few typical examples are cited in the following Table 9.2.

Table 9.2 : Substances Assayed by Direct Titration with Disodium-EDTA

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Calcium carbonate	0.1 g	Calcon mixture	Each ml of 0.05 M disodium edetate ≡ 0.005004 g of $CaCO_3$
2.	Dibasic calcium phosphate	0.2 g	Hydroxy naphthol blue	Each ml of 0.05 M disodium edetate ≡ 0.002004 g of Ca
3.	Magnesium chloride	0.5 g	Mordant black II mixture	Each ml of 0.05 M disodium edetate ≡ 0.017017 g of $MgCl_2.6H_2O$
4.	Heavy magnesium oxide	0.1 g	Mordant black II mixture	Each ml of 0.05 M disodium edetate ≡ 0.002015 g of MgO
5.	Magnesium trisilicate (for MaO)	1.0 g	-do-	Each ml of 0.05 M disodium edetate ≡ 0.002015 g of MgO

6.	Zinc chloride	3.0 g	Eriochrome Black-T	Each ml of 0.05 M disodium edetate ≡ 0.006815 g of $ZnCl_2$
7.	Zinc stearate	1 .0 g	-do-	Each ml of 0.05 M disodium edetate ≡ 0.004069 g of ZnO
8.	Zinc sulphate	0.3 g	-do-	Each ml of 0.05 M disodium edetate ≡ 0.01438 g of $ZnSO_4.7H_2O$
9.	Zinc undecylenate	0.5 g	-do-	Each ml of 0.05 M disodium edetate ≡ 0.02160 g of $C_{22}H_{38}O_4Zn$

9.3.2. MASKING AND DEMASKING AGENTS

The disodium ethylenediaminetetracetate usually complexes with a wide spectrum of cations, which ultimately renders the selectivity of the titration procedure adversely, thereby providing enough scope for the accompanying metal impurities to be titrated along with the ion it is aimed at for actual estimation. Therefore, in a situation where one or two ions present in a mixture of cations is specifically required to be determined with a view to eliminate completely the possible effects of unwanted impurities that may enhance the titre value, a third substance is added, which is known as the Masking Agent. These agents must fulfil the following *three* requirements, namely :

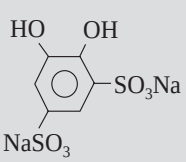
(*a*) should act by precipitation,

(*b*) should form complexes that are definitely more stable than the interfering ion-edetate complex, and

(*c*) colour developed by either precipitates or auxiliary complexes should not obscure the end-point.

A few typical examples are cited below in Table 9.3 where masking has been accomplished by precipitation.

Table 9.3 : Masking Accomplished by Precipitation

S.No.	Interfering Heavy Metal Ions	Complexing Agent	Remarks
1.	Co^{2+}, Cu^{2+}, Pb^{2+}	Na_2S (sodium sulphide), CH_3CSNH_2 (Thioacetamide)	As insoluble sulphides and complexes
2.	Cu^{2+}	$HS.CH_2.CHOH.CH_2OH$ (Thioglycerol)	As insoluble complex
3.	Al^{3+}, Fe^{3+}, Ti^{3+}	NH_4F (Ammonium fluoride)	Complex formation
4.	Hg^{2+}, Cd^{2+}, Zn^{2+}, As^{3+}, Sb^{3+}, SN^{4+}, Pb^{2+}, Bi^{2+}	$HSCH_2.CHSH.CH_2OH$ (Dimercaprol)	Precipitation in weakly acidic medium while soluble in alkaline medium
5.	Hg^{2+}	KI (Potassium iodide)	Masks Hg^{2+} as HgI_4^{4-}
6.	Al^{3+}, Ti^{3+}	 (Disodium catechol-3, 5-disulphonate)	Forms colourless complexes
7.	Al^{3+}, Fe^{3+}, Mn^{3+}	$[N(CH_2CH_2OH)_3]$ (Triethanolamine)	Al-complex : colourless ; Fe-complex : Yellow ; Mn-complex : Green

9.3.3. RESIDUAL TITRATION METHODS

Direct titration method offers a serious limitation for the assay of aluminium and bismuth containing pharmaceutical inorganic substances because of the precipitation of the metal as their corresponding hydroxides in alkaline media thereby introducing undesirable errors.

In actual practice, an excess of the standard solution of disodium edetate is added to the sample, pH is adequately adjusted for the residual titration with a metal-ion solution *e.g.*, $ZnSO_4$ and employing an appropriate indicator which is sensitive enough to the respective titrant. However, the metal ion under estimation remains firmly complexed with the EDTA and offers little interference with the Zn-EDTA complex formed. It has been established experimentally that bismuth readily yields a highly stable complex which may be titrated conveniently between pH 1 and 2. Bismuth forms a stable complex by reacting with EDTA quantitatively at pH 4.0 and, therefore, dithizone is employed as an indicator to detect the end-point for it has a transition state of colour at pH 4.6.

9.3.3.1. Potassium Alum, $KAl(SO_4)_2$, $12H_2O$

Materials Required : Potassium alum : 1.7 g ; 0.05 M disodium edetate : 30.0 ml ; hexamine : 1.0 g ; 0.05 M lead nitrate ; xylenol orange solution (0.1% w/v in water) : 0.4 ml.

Theory : The solution of potassium alum is heated with an excess of disodium edetate to ensure complete formation of aluminium-edetate complex. Hexamine serves as a buffer thereby stabilizing the pH between 5 and 6, the ideal pH for the titration of the disodium edetate not required by the Al with 0.05 M lead nitrate employing xylenol orange as indicator. The various reactions involved may be represented by the following equations :

$$Al^{3+}\ [H_2X]^{2-} \longrightarrow [AlX]^- + 2H^+$$

or $$KAl(SO_4)_2,\ 12H_2O \equiv Al^{3+} \equiv Na_2H_2X,\ 2H_2O$$

or 474.4 g $KAl(SO_4)_2,\ 12H_2O \equiv$ 20,000 ml 0.05 M

or 0.02372 g $KAl(SO_4)_2,\ 12H_2O \equiv$ 1 ml of 0.05 M Disodium Edetate

Procedure : Weigh accurately 1.7 g of potassium alum and dissolve it in suffcient DW in a flask. Heat the contents of flask over a water-bath for 10 minutes to allow completion of complexation and cool to ambient temperature. Now, add 1 g hexamine to act as buffer and titrate with 0.05 M lead nitrate employing 0.4 ml of xylenol orange solution as an indicator. The colour shall change from that of the indicator (yellow at the pH of the titration) to the corresponding reddish purple, the colour of the lead complex of the indicator. Each ml of 0.05 M disodium edetate is equivalent to 0.02372 g of $KAl(SO_4)_2,\ 12H_2O$.

9.3.3.2. Glycobiarsol [Bismethyl-N-glycolyl-arsanilate]

Materials Required : Glycobiarsol : 0.2 g ; 0.05 M disodium edetate : 10.0 ml ; acetic acid-ammonium acetate buffer (mix 13.6 g of sodium acetate and 7.7 g of ammonium acetate in water to make 100 ml. Add 25.0 ml of glacial acetic acid and mix) : 10.0 ml ; alcohol : 25.0 ml : dithizone solution (0.05% w/v in chloroform) : 2.0 ml ; 0.025 M $ZnSO_4$ solution.

Procedure : Weigh accurately 0.20 g of glycobiarsol into a 250-ml conical flask and add 10.0 ml of 0.05 M disodium edetate. Warm the contents of the flask over a water-bath until glycobiarsol gets dissolved completely and then cool the contents to the room temperature (25°C). Add to it 10.0 ml of acetic acid-ammonium acetate buffer, 25.00 ml of alcohol and 2 ml of dithizone solution as an indicator. Titrate the excess of disodium edetate with 0.025 M zinc sulphate until the resulting solution turns rose pink in colour. Each millilitre of 0.05 M disodium edetate consumed is equivalent to 10.45 mg of Bi.

Note : The content of Bi, calculated on dried basis, lies between 38 to 42.5%.

9.3.3.3. Cognate Assays

A number of inorganic pharmaceutical substances may be assayed by adopting the residual titration method as depicted in Table 9.4.

Table 9.4 : Substances Assayed by Residual Titration with EDTA

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Aluminium glycinate	0.25 g	Methyl Red	Each ml of 0.05 M disodium edetae ≡ 0.002549 g of Al_2O_3
2.	Dried Aluminium Hydroxide	0.8 g	-do-	Each ml of 0.1 M disodium edetate ≡ 0.005098 g of Al_2O_3
3.	Aluminium sulphate	0.5 g	-do-	Each ml of 0.1 M disodium edetate ≡ 0.01711 g of $Al_2(SO_4)_3$
4.	Bismuth subcarbonate	0.5 g	-do-	Each ml of 0.1 M disodium edetate ≡ 0.02090 g of Bi

THEORETICAL AND PRACTICAL EXERCISES

1. What is the underlying principle of '**Complexometric titrations**' ? Give appropriate examples in support to your answer.

2. Discuss the following aspects in an elaborated fashion :

(*i*) Number of '**rings**' formed in the complex with bivalent, trivalent and tetravalent metal ions.

(*ii*) Effect of pH on complexation

(*iii*) Stability of complexes

(*iv*) Usage of pM indicators in complexometric titrations

(*v*) Titrability of polyvalent metal ions employing disodium EDTA.

3. How would your carry out complexometric titrations by the '**direct titration method**' ? Discuss the assay of the following pharmaceutical drugs explicitely :

(*i*) Magnesium sulphate (*ii*) Calcium carbonate

(*iii*) Dibasic calcium phosphate (*iv*) Zine undecylenate.

4. What are 'masking and demasking agents' with reference to complexometric titrations ? Give specific examples to justify your statements.

5. How does '**residual titration method**' help in the complexometric titrations ? Elaborate the assay of the following drugs by this technique :

(*i*) Potassium alum (*ii*) Bismuth subcarbonate

(*iii*) Aluminium glycinate (*iv*) Dried aluminium hydroxide.

RECOMMENDED READINGS

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E. GRAVIMETRIC METHODS

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10 GRAVIMETRIC ANALYSIS

CONTAINS :

10.1 Introduction
10.2 Theory
10.2.1 Law of mass actions of reversible reactions
10.2.2 Principle of solubility product
10.2.3 Common ion effect
10.3 Assay Methods
10.3.1 Substances assayed gravimetrically
10.3.2 Substances assayed after conversion

10.1. INTRODUCTION

Gravimetric analysis is an unique technique by means of which either an element or a compound is obtained in its purest form through isolation and subsequent weighing. In order to achieve this, the element or compound is first and foremost separated from a specific portion of the pharmaceutical substance being determined and consequently the weight of the constituent in the given sample is calculated on the basis of the weight of the product.

However, in actual gravimetric analysis, the final weight of the product is usually accomplished by adopting anyone of the following standard methods, namely :

(*a*) Solvent extraction,

(*b*) Ignition or volatalization, and

(c) Precipitation from solution.

Gravimetric techniques are broadly based upon the quantitative precipitation of the respective cation or anion from a given solution in *two* different ways :

(*i*) as an insoluble compound that yields a residue having a specific composition after ignition, and

(*ii*) as an insoluble compound having a known composition.

There are *four* vital steps that are essentially required for a successful gravimetric method, namely :

(*a*) Identify an insoluble form with a definite composition,

(*b*) Separate the analyte exclusively from another constituents which may cause interference,

(*c*) Wash the precipitate free of coprecipitants and impurities as far as possible, and

(*d*) Convert the precipitate ultimately to a reasonably measurable form.

10.2. THEORY

The underlying principles and theories of gravimetric analysis are as stated below :

(*i*) Law of mass action and reversible reactions,

(*ii*) Principle of solubility product, and

(*iii*) Common ion effect.

All the above three aspects shall be described briefly *vis-a-vis* their direct impact on the gravimetric analysis.

10.2.1. LAW OF MASS ACTION AND REVERSIBLE REACTIONS

A plethora of chemical reactions that are intimately associated with the quantitative analysis essentially belong to the class of reversible reactions. These reactions under certain prevailing experimental parameters are made to proceed to completion, whereas in certain other conditions they may even attain equilibrium before completion. In the latter instance, erroneous results may creep in with regard to the pharmaceutical substance under estimation. Hence, it has become absolutely necessary first to establish the appropriate conditions whereby the reactions must move forward to attain completion so as to achieve the ultimate objective in all quantitative assays.

In general, there are *three* cardinal experimental parameters that must be observed rigidly in order to check the reversal processes and help the completion of a reaction, namely :

(*a*) formation of very slightly ionized molecules,

(*b*) formation of an insoluble gas, and

(*c*) formation of a sparingly soluble solid.

The '**law of mass action**' advocates that the rate of a reaction is directly proportional to the product of the molecular concentrations of the reacting substances. For example :

$$BaCl_2 + H_2SO \rightleftharpoons BaSO_4 + 2HCl$$

In the above reaction the rate of reaction of barium chloride with sulphuric acid is designated by the following expression :

Forward reaction :

$$\text{Rate} = [BaCl_2] \times [H_2SO_4] \times k \qquad ...(a)$$

where, k = a constant that corrects for all factors which affect the rate other than concentration.

Likewise, in the opposing reaction, we have :

Opposing reaction :

$$\text{Rate} = [BaSO_4] \times [HCl] \times k_1 \qquad ... (b)$$

where, k_1 = another constant.

At equilibrium the rates of the forward reaction (*a*) and opposing reaction (*b*) are equal. Hence, we have :

$$[BaCl_2] \times [H_2SO_4] \times k = [BaSO_4] \times [HCl] \times k_1 \qquad ...(c)$$

Rearranging (*c*) we have :

$$\frac{[BaCl]_2 \times [H_2SO_4]}{[BaSO_4][HCl]} = \frac{k}{k_1} = K \qquad ... (d)$$

As k and k_1 are constants, their quotient K is also a constant known as the equilibrium constant.

From Eq. (*d*), K, the equilibrium constant has a fixed value at a definite temperature, irrespective of concentrations of other components present.

Therefore, if the concentration of sulphuric acid is enhanced, consequently all other concentrations should change accordingly, the concentration of $BaCl_2$ must become less and that of both $BaSO_4$ and HCl be proportionately greater so as to maintain the equilibrium constant, thereby having the net impact of shifting the equilibrium towards the right hand side. Evidently, in most quantitative analysis one entity is added invariably to allow the reaction to proceed as closely to completion as possible.

10.2.2. PRINCIPLE OF SOLUBILITY PRODUCT

The principle of solubility product may be stated as follows :

'*The product of the concentration of the constituent ions in a saturated solution of a difficultly soluble salt for any given temperature is practically a constant, each concentration being raised to a power equal to the relative number of ions supplied by one molecule of the salt upon dissociating*'.

The principle of solubility product is applicable to :

(*i*) difficultly soluble salts in their saturated solutions,

(*ii*) occurrence of precipitation,

(*iii*) prevention of precipitation, and

(*iv*) dissolution of a substance.

For instance, a difficultly soluble salt A*p*B*q* on dissociation provides a relative number of *p* cations and *q* anions. Thus, we have :

$$A_pB_q \rightleftharpoons pA^+ + qB^-$$

Hence, solubility product $A_pB_q = [A^+]^p \times [B^-]^q$

where, [ ] are generally used to express the molar concentrations.

Table 10.1, contains the solubility products of certain difficultly soluble salts generally encountered in pharmaceutical analysis.

Table 10.1 : Solubility Products of Important Inorganic Salts

S.No.	Name of Substance	Temp. °C	Ions Involved	Solubility Product
1.	Aluminium hydroxide	*a*	$Al^{3+} + 3OH^-$	1×10^{-33}
2.	Barium sulphate	*a*	$Ba^{2+} + SO_4^{2-}$	1.1×10^{-10}
3.	Calcium oxalate	*a*	$Ca^{2+} + C_2O_4^{2-}$	2.6×10^{-9}
4.	Lead sulphate	*b*	$Pb^{2+} + SO_4^{2-}$	1.1×10^{-8}
5.	Magnesium oxalate	*b*	$Mg^{2+} + C_2O_4^{2-}$	8.8×10^{-5}
6.	Mercuric sulphide	*a*	$Hg^{2+} + S^{2-}$	1×10^{-50}
7.	Silver chloride	*a*	$Ag^+ + Cl^-$	1.5×10^{-10}
8.	Silver thiocyanate	*a*	$Ag^+ + SCN^-$	1.2×10^{-12}

a = 25°C ; *b* = 18°C.

The interaction of $AgNO_3$ and NaCl results into the formation of AgCl which is slightly soluble in water, the solubility being approximately 0.00001 ml litre^{-1} *i.e.*, 1.5 mg litre^{-1}. On exceeding this concentration, the AgCl gets precipitated which remains in equilibrium with the dissolved AgCl. Therefore, at equilibrium, the clear supernatant liquid is a saturated solution, and at this critical juncture the rate at which the dissolved salt gets precipitated is almost equal to the rate at which the solid undergoes dissolution. This establishes the following equilibria :

$$\underset{\text{Solid precipitate}}{AgCl} \rightleftharpoons \underset{\text{Dissolved unionized}}{AgCl} \rightleftharpoons \underset{\text{Dissolved ionized}}{Ag^+ + Cl^-}$$

Hence, the ionization equilibrium may be expressed as follows :

$$\frac{[Ag^+] \times [Cl^-]\ \text{ionized}}{AgCl\ \text{unionized}} = K$$

where, K = ionization constant.

Considering the following *two* assumptions :

(*i*) solution remains saturated with AgCl at a aiven temperature, and

(*ii*) concentration of unionized AgCl remains constant, it follows, that the product K × [AgCl] too becomes constant.

Therefore, it may be inferred that—'*in a saturated solution of a difficultly soluble salt, the product of the molecular concentration of its ions is constant*'.

For instance :

(*a*) $BaSO_4 \rightleftharpoons Ba^{2+} + SO_4^{2-}$

$[Ba^{2+}] \times [SO_4^{2-}]$ = Solubility product of $BaSO_4$

(*b*) $CaC_2O_4 \rightleftharpoons Ca^{2+} + C_2O_4^{2-}$

$[Ca^{2+}] \times [C_2O_4^{2-}]$ = Solubility product of CaC_2O_4

(*c*) $Al(OH)_3 \rightleftharpoons Al^{3+} + 3OH^-$

$[Al^{3+}] \times [OH^-]\ 3$ = Solubility product of $Al(OH)_3$

It is an usual practice to express the concentration of the solubility product in terms of moles per litre *i.e.*, molar concentrations.

10.2.3. COMNION ION EFFECT

It has been observed that there is no change in the equilibrium constant even if :

(*a*) the concentrations of reacting components may change, and

(*b*) the relative concentration of the reacting substances may change.

When a solution of $BaCl_2$ is added to a solution of sulphuric acid, the sulphate ion for a while is present in a concentration in such a manner that its ionic product with the barium ion exceeds the solubility product of barium sulphate, and the insoluble barium sulphate gets precipitated :

$$Ba^{2+} + SO_4^{2-} \longrightarrow BaSO_4 \downarrow$$

However, at equilibrium the concentration of Ba^{2+} ions shall be exactly equal to the concentration of sulphate ions.

Now, if to the resulting supernatant liquid, which is nothing but a saturated solution of barium sulphate, an additional small quantity of either a soluble barium salt or a soluble sulphate is provided, a slight further precipitation may occur.

Hence, the equilibrium that represents the ionization constant may be expressed as :

$$\frac{[Ba^{2+}] \times [SO_4^{2-}]}{[BaSO_4]} = K \qquad \ldots (a)$$

From Eq. (*a*), it may be derived that if the concentration of Ba^{2+} ion is enhanced by the addition of a soluble barium salt, the concentration of sulphate ion should decrease simultaneously and conversely, that if the concentration of sulphate ion is enhanced by the addition of a soluble sulphate salt, the concentration of Ba^{2+} ion should decrease as their product almost remains constant. Evidently, this decrease in the concentration of the ions in either instance may be achieved by the combination of barium and sulphate ions to give rise to the insoluble barium sulphate thereby forcing the reaction towards completion.

In short, the **common-ion effect** is employed invariably in carrying out the gravimetric analysis of pharmaceutical substances so as to drive reactions toward completion.

Calculations : In gravimetric analysis the percentage of the desired constituent may be achieved by the following expression :

$$\text{Percentage of desired constituent} = \frac{\text{Wt. of precipitate} \times \text{Gravimetric factor}}{\text{Wt. of sample}} \times 100$$

The term '**gravimetric factor**' is generally employed which represents the number of grams of the desired constituent in 1 g of the substance weighed. It can be further expatiated with the help of the following examples :

(*i*) One mole of $BaSO_4$ (233.39 g) contains one mole of SO_4 atoms (96.06 g).

$$\text{Hence, the Gravimetric Factor} = \frac{SO_4}{BaSO_4} = \frac{96.06}{233.39} = \mathbf{0.4116}$$

(*ii*) One mole of AgCl (143.323 g) contains one mole of Cl atoms (35.453 g).

$$\text{Hence, the Gravimetric Factor} = \frac{Cl}{AgCl} = \frac{35.453}{143.323} = \mathbf{0.2474}$$

10.3. ASSAY METHODS

A good number of pharmaceutical substances can be assayed gravimetrically. The gravimetric methods adopted vary according to the nature of the substance under determination. However, most of the substances being estimated gravimetrically fall into one or the other categories stated below, which would be discussed briefly with suitable examples :

(*a*) Substances assayed gravimetrically,

(*b*) Substances assayed after conversion :

(*i*) Substances assayed after conversion to Free Acid,

(*ii*) Substances assayed after conversion to Free Base,

(*iii*) Substances assayed after conversion to Free Compound, and

(*iv*) Substances assayed after conversion to Derivatives or Substitution Products.

10.3.1. SUBSTANCES ASSAYED GRAVIMETRICALLY

A good number of pharmaceutical substances may be determined gravimetrically by obtaining their respective difficultly soluble salts as precipitates, weighing to a constant weight and finding the percentage purity of the substance in question.

A few typical examples are cited below so as to expatiate the procedure as well as the theoretical aspects.

10.3.1.1. Sodium Chloride

Materials Required : Sodium chloride : 0.25 g ; 5% w/v silver nitrate in DW (+ 2-3) drops of conc. HNO_3 ; dilute nitric acid (6 N) ; asbestos fibre.

Theory : The following reaction forms the basis for the calculation of the theoretical amount of silver nitrate solution required as well as the purity of the given sample of NaCl. Thus, we have :

$$\underset{169.87}{AgNO_3} + \underset{58.44}{NaCl} \longrightarrow \underset{143.22}{AgCl} + \underset{84.99}{NaNO_3}$$

Therefore, $NaCl \equiv AgNO_3$

or $$1 \text{ g of NaCl} \equiv \frac{169.87}{58.44} \equiv 2.9067 \text{ g of } AgNO_3$$

As 0.2570 g of NaCl has been used (from experimental data); therefore, the exact amount of $AgNO_3$ required would be :

$$0.2570 \times 2.9067 = 0.7470 \text{ g of } AgNO_3$$

(considering NaCl to be 100% pure)

The $AgNO_3$ solution is 5% w/v :

or $$1 \text{ ml of } 5\% \ AgNO_3 \equiv 0.05 \text{ g of } AgNO_3.$$

Hence, the amount of $AgNO_3$ solution required theoretically would be 0.7470/0.05 = 14.94 ml.

From above, the percentage purity of the given sample of NaCl may be found as shown below :

$$\frac{58.44}{143.22} = 0.4078 \text{ g of NaCl} \equiv 1 \text{ g AgCl}$$

The weight of AgCl is found to be 0.6288 g experimentally, or 0.4078 is the 'gravimetric factor'.

Consequently, the percentage purity of the sample is determined by the formula :

$$\frac{W \times E \times 100}{S} = \%$$

where, W = Wt. of the product of a chemical reaction with the substance under determination,

E = Gravimetric Factor, and

S = Wt. of the sample.

By incorporating the data given above, the amount of sodium chloride present in 100 g of the sample *i.e.*, the percentage purity of NaCl in the given sample may be calculated as follows :

$$\frac{0.6288 \times 0.4078 \times 100}{0.2570} = 99.77\%$$

Procedure : Weigh accurately between 0.20 to 0.30 g of sodium chloride and dissolve in 100 ml of DW. Add to it 1 ml of dilute nitric acid gradually with constant stirring. Check and confirm that the resulting solution is acidic with the help of blue litmus paper. Measure out 5.0 ml in excess of the amount of silver nitrate solution calculated on theoretical basis to precipitate all the available chlorine as silver chloride. The requisite quantity of silver nitrate solution must be added in small lots at intervals with constant stirring with a glass rod. Cover the beaker with a watch-glass and boil the contents very gently with occasional stirring (to avoid bumping of the liquid and loss of volume). Stop heating and digest the mixture for 10 minutes so as to agglomerate the precipitate and enhance settling thereby leaving a clear supernatant liquid. Add 2 drops of silver nitrate solution to the hot supernatant liquid in order to confirm whether precipitation is completed. Keep the beaker away from direct sunlight to allow the precipitate to settle.

Take a properly prepared Gooch crucible, heat to constant weight and fit it into the suction flask. Decant most of the supernatant liquid first into the Gooch crucible by applying gentle suction to hasten filtration. Wash the precipitate on the Gooch crucible at least thrice with 15 ml portions of 0.01 N nitric acid.

Test the above filtrate to be free of $AgNO_3$. Finally wash the precipitate twice with 5 ml portion of DW to get rid of most of the HNO_3 previously retained by the precipitate from the former wash solution. Now, apply vigorous suction to drain out the liquid from the precipitate to the maximum extent. Dry the crucible to a constant weight between 110-120°C in an electric oven until two concurrent weighings are achieved. Thus, the weight of the crucible (tare) must be deducted from the weight of the crucible plus the precipitate to arrive at the weight of silver chloride duly obtained from the sample.

Precautions :

(1) The solution of the substance is usually acidified with HNO_3 to check the precipitation of other substances insoluble in water but soluble in HNO_3 *e.g.*, CO_3^{2-}, O^{2-} and PO_4^{3-}. Besides HNO_3 also helps to coagulate any colloidal AgCl,

(2) The excess of HNO_3 must be avoided to cause solvolysis of silver halides,

(3) Heating should be affected only after the addition of $AgNO_3$, otherwise Cl_2 may be liberated and lost. Thus, we have :

$$NaCl + HNO_3 \longrightarrow HCl + NaNO_3$$

$$6HCl + 2HNO_3 \longrightarrow 3Cl_2 \uparrow + 4H_2O + 2NO$$

(4) The precipitation should preferably be carried out in the absence of strong light because AgCl undergoes decomposition in sunlight with loss of Cl_2,

(5) Washing of the precipitate (AgCl) with 0.01 N HNO_3 is always recommended to prevent loss of AgCl by virtue of its return to colloidal condition (peptization) and to get rid of the soluble salts, namely : AgNO and $NaNO_3$, and

(6) AgCl is significantly volatile on ignition, hence it must always be dried at a comparatively lower temperature.

10.3.1.2. Potassium Alum, $KAl(SO_4)_2$, $12H_2O$

Theory : The percentage of Al in potassium alum can be determined volumetrically by complexometric titration (see Chapter : 9).

However, gravimetric procedure provides a fairly reliable and useful alternative method of analysis for Al which may be accomplished by :

(*a*) precipitation from a solution of the aluminium salt by the addition of NH_4OH in the presence of NH_4Cl, and

(*b*) complexation from a solution of the aluminium salt with 8-hydroxyquinoline (oxine) either from an ammoniacal solution or from acetic acid-acetate buffer.

In the first method, the following reaction takes place :

$$Al^{3+} + 3OH^- \longrightarrow Al(OH)_3 \downarrow$$

The gelatinous white precipitate of $Al(OH)_3$ is duly filtered, washed with dilute NH_4NO_3 solution, transformed to the corresponding oxide and finally weighed as Al_2O_3 .

Disadvantages : There are a number of serious disadvantages of this method, namely :

(*i*) excess of NH_4OH may directly affect the solubility of $Al(OH)_3$,

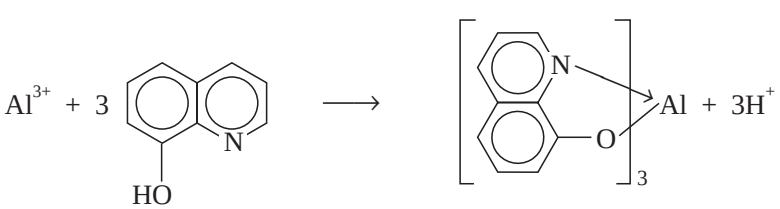
(*ii*) coprecipitation of metal hydroxides that are usually soluble in NH_4OH,

(*iii*) heated oxide (Al_2O_3) is hygroscopic in nature, and

(*iv*) hydroxides may not undergo complete thermal decomposition.

Due to the above short-comings, the second method is usually preferred which shall be discussed below :

Equation :



(Oxine)

Aluminium-oxine complex
(Aluminium-oxinate)

The resulting precipitate of aluminium-oxine complex is crystalline in nature and hence can be filtered conveniently, washed with water and finally dried at 130-150°C to constant weight.

Disadvantages : There are *two* disadvantages of the metal-oxine-complex method, namely :

(*i*) aluminium-oxinate is prone to adsorb oxine, and

(*ii*) lack of selectivity of oxine such that all metals except the alkaline earths (Ba, Mg, Ca, Sr, Be) and alkali (Li, Na, K, Rb, Cs) should be totally absent.

Calculations :

$$KAl(SO_4)_2, 12H_2O \equiv Al(C_9H_6NO)_3$$

or $$26.98 \text{ g Al} \equiv 459.4 \text{ g } Al(C_9H_6NO)_3$$

or $$0.05873 \text{ g Al} \equiv 1 \text{ g of } Al(C_9H_6NO)_3$$

Materials Required : Potassium alum : 0.3 g ; 0.1 N hydrochloric acid : 1.0 ml ; 8-hydroxyquinoline reagent (or oxine-reagent) (25 ml of a 2% w/v solution of oxine in 2 N acetic acid) ; 2 N ammonium acetate (dissolve 15.0 g of ammonium acetate in 20.0 ml of DW, add 0.3 ml of glacial acetic acid and dilute to 100 ml with DW) ; sintered glass crucible No : 3 or 4.

Procedure : Weigh accurately about 0.3 g of potassium alum in a 400-ml beaker, dissolve it in 150 ml of DW containing 1.0 ml of 0.1 N HCl and warm the contents of the beaker to about 60°C. Add the requisite quantity of the oxine reagent and then add a 2 N solution of ammonium acetate gradually from a pipette till precipitation just commences. Add a further portion (50 ml) of ammonium acetate solution with vigorous stirring. Allow the contents of the beaker to stand for 60 minutes with frequent stirring. Filter the precipitate through No : 3 or 4 sintered glass crucible that has been previously dried to a constant weight at 130—150°C. Wash the precipitate throughly with cold DW and dry at 130 to 150°C to constant weight. Each gram of aluminium oxinate is equivalent to 0.05873 g of Al.

10.3.1.3. Cognate Assays

A good deal of pharmaceutical substances are officially assayed gravimetrically as appears in Table 10.2.

Table 10.2 : Substances Assayed Gravimetrically

S.No.	Name of Substance	Qty. Prescribed	Drying Temp. (°C)	Calculations
1.	Barium sulphate	0.60 g	105	Each g of the residue ≡ 0.9213 g of $BaSO_4$
2.	Fluorescein sodium	0.50 g	105	Each g of the residue ≡ 1.132 g of $C_{20}H_{10}Na_2O_5$
3.	Piperazine adipate	0.20 g	105	Each g of the residue ≡ 0.4268 g of $C_4H_{10}N_2$, $C_6H_{10}O_4$
4.	Piperazine hydrate	0.20 g	105	Each g of the residue ≡ 0.3567 g of $C_4H_{10}N_2$, 6 H_2O
5.	Piperazine phosphate	0.20 g	105	Each g of residue ≡ 0.3382 g of $C_4H_{10}N_2$, H_3PO_4
6.	Piperazine phosphate Tabs.	0.15 g	105	Each g of the residue ≡ 0.3714 g of $C_4H_{10}N_2$, H_3PO_4, H_2O
7.	Quinalbarbitone Tablets	0.10 g	105	Each g of the residue ≡ 1.092 g of $C_{12}H_{17}N_2NaO_3$
8.	Quiniodochlor Tablets	0.10 g	105	Each g of the residue ≡ 0.91 g of C_9H_5ClNO
9.	Sodium aurothiomalate (For Na)	0.2 g	600	Each g of the residue ≡ 0.03237 g of Na
10.	Sulphobromophthalein sodium (For Sulphur)	0.2 g	600	Each g of the residue ≡ 0.1374 g of S
11.	Thiocarbazone	0.1 g	105	Each g of the residue ≡ 0.4606 g of $C_{10}H_{12}N_4OS$

10.3.2. SUBSTANCES ASSAYED AFTER CONVERSION

There are certain pharmaceutical substances that can be assayed gravimetrically after their suitable conversion to free acid, or free base, or free compound or corresponding derivatives (or substitution products). All these typical cases shall be discussed briefly with their appropriate examples in the following sections.

10.3.2.1. Substances Assayed after Conversion to Free Acid

A few official pharmaceutical substances may be assayed gravimetrically by affecting separation, purification, and weighing an organic medicinal compound without causing any permanent change in composition. It is an usual practice that before extraction of the organic medicinal compound, the sample of the crushed tablets is carefully washed with petroleum benzene to get rid of undesirable components, for instance : lubricants and binders that would be extracted along with the organic medicinal compound by such solvents as ether or chloroform which is employed subsequently.

In case, the organic medicinal compound is acidic in nature *e.g.*, amobarbital in sodium amobarbital tablets, it is first and foremost extracted with an aqueous solution of an acid or base to cause separation from the neutral substance which might be present. The resulting aqueous solution of the salt of the respective organic medicinal compound is subsequently made acidic and the liberated organic acid (amobarbital) is finally extracted with ether or chloroform.

Interestingly, in a situation where either magnesium stearate or stearic acid forms a component in the formulation, the organic medicinal compound which is acidic (amobarbital) cannot be extracted with NaOH solution for obvious reason that sodium stearate shall also be extracted along with the salt of the organic acid. Therefore, instead a saturated solution of $Ba(OH)_2$ is employed thereby the insoluble precipitate of barium stearate may be discarded by filtration.

10.3.2.1.1. *Phenobarbitone Sodium*

Materials Required : Phenobarbitone sodium : 0.5 g ; hydrochloric acid (2 M) : (dissolve 17.0 ml ($\simeq$ 11.5 N) in 100 ml DW) : 5.0 ml ; ether : 13.5 ml ; absolute ethanol : 2.0 ml.

Procedure : Weigh accurately 0.5 g phenobarbitone sodium and dissolve in 15 ml of DW. Add to it 5 ml of 2 M hydrochloric acid and extract with 50 ml of ether and then with successive 25 ml quantities of ether until complete extraction is affected. Wash the combined extracts with two 5 ml quantities of DW and wash the combined aqueous extracts with 10 ml quantities of ether. Add the ether to the main ethereal extract, evaporate to low bulk, add 2 ml of absolute ethanol, evaporate to dryness and dry the residue to constant weight at 105°C. Each g of residue is equivalent to $C_{12}H_{11}N_2NaO_3$.

Calculations :

$$C_{12}H_{11}N_2NaO_3 \equiv C_{l2}H_{l2}N_2O_3$$

or $$254.2 \text{ g } C_{12}H_{11}N_2NaO_3 \equiv 232.2 \text{ g } C_{12}H_{12}N_2O_3$$

or $$1.095 \text{ g } C_{l2}H_{11}N_2NaO_3 \equiv 1 \text{ g of } C_{l2}H_{12}N_2O_3$$

10.3.2.1.2. *Cognate Assays*

There are certain pharmaceutical substances that may be assayed after their conversion to the respective free acids as shown in Table 10.3.

Table 10.3 : Substances Assayed Gravimetrically by Conversion to Free Acid

S.No.	Name of Substance	Qty. Prescribed	Drying Temp. (°C)	Calculations
l.	Amobarbital sodium	0.5 g	l05	Each g of residue ≡ 1.097 g of $C_{11}H_{17}N_2NaO_3$
2.	Pentobarbital sodium Tablets	0.3 g	105	Each g of residue ≡ 0.1097 g of $C_{11}H_{17}N_2NaO_3$
3.	Phenytoin sodium	0.3 g	105	Each g of residue ≡ 1.087 g of $C_{l5}H_{11}N_2NaO_2$
4.	Secobarbital sodium	0.5 g	100	Each g of the residue ≡ 1.092 g of $C_{12}H_{17}N_2NaO_3$

10.3.2.2. Substances Assayed after Conversion to Free Base

In a specific instance where the organic medicinal substance is basic in nature *e.g.*, papaverine in papaverine hydrochloride, it is primarily treated with an aqueous solution of a base and subsequently the liberated organic base is extracted with either chloroform or ether.

A typical example is described below :

10.3.2.2.1. *Papaverine Hydrochloride Tablets*

Materials Required : Sodium hydroxide (2 M) (dissolve 8.0 g of NaOH pellets in 100 ml of CO_2 free DW : 50 ml ; chloroform : 100 ml ; absolute ethanol : 5 ml.

Calculations :

$$C_{20}H_{21}NO_4 \,.\, HCl \equiv C_{20}H_{21}NO_4$$

or $$374.45 \text{ g } C_{20}H_{21}NO_4 \,.\, HCl \equiv 339.0 \text{ g } C_{20}H_{21}NO_4$$

or $$1.105 \text{ g } C_{20}H_{21}NO_4 \,.\, HCl \equiv 1 \text{ g of } C_{20}H_{21}NO_4$$

Procedure : Weigh 20 tablets and crush them in a pestle mortar and find out the average weight of a single tablet. Accurately weigh 0.5 g equivalent of papaverine hydrochloride and dissolve in 15 ml of DW. Add to it 15 ml of 2 M sodium hydroxide and extract with 50 ml of chloroform and then with successive 25 ml quantities of chloroform until complete extraction is affected. Wash the combined extracts with two 5 ml quantities of DW and wash the combined aqueous extract with two 10 ml quantities of chloroform. Add the chloroform to the main chloroform extract, evaporate to a small volume, add 2 ml of absolute ethanol, evaporate to dryness and dry the residue to constant weight at 105°C.

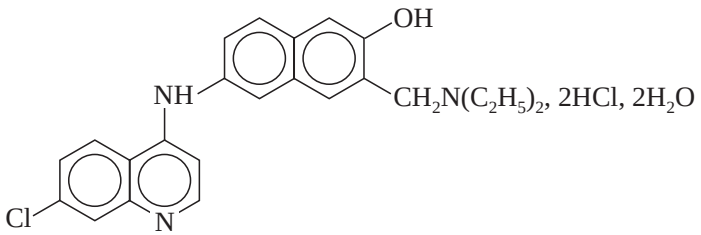
Each g of the residue is equivalent to 1.105 g of $C_{20}H_{21}NO_4$. HCl.

10.3.2.2.2. *Amodiaquine Hydrochloride*

Materials Required : Amodiaquine hydrochloride : 0.3 g ; dilute ammonia solution (42.5 ml of strong ammonia solution to 100 ml in water) ; NO. 4 sintered glass crucible.

Theory : Amodiaquine hydrochloride possesses two moles of inherent water of crystallization, and hence the precentage base is calculated with reference to the substance dried over P_2O_5 at a pressure not exceeding 5 mm of Hg. Usually, the assay is performed on one portion of the sample and the drying on a separate portion altogether.

The underlying principle of the method is based upon the precipitation of amodiaquine base that is generated as a precipitate when the salt is decomposed in aqueous medium with dilute ammonia.



$$C_{20}H_{22}ON_3Cl,\ 2HCl,\ 2H_2O + 2NH_3 \longrightarrow \underset{\text{Amodiaquine base}}{C_{20}H_{22}ON_3}\downarrow + 2NH_4Cl + 2H_2O$$

or 464.35 g $C_{20}H_{22}ON_3Cl$, 2HCl, $2H_2O \equiv$ 355.4 g $C_{20}H_{22}ON_3$

or 1.306 g $C_{20}H_{22}ON_3Cl$, 2HCl, $2H_2O \equiv$ 1 g of $C_{20}H_{22}ON_3$

Procedure : Weigh accurately 0.3 g of previously dried amodiaquine hydrochloride into a 100 ml beaker provided with a stirring rod and watch glass cover. Dissolve it in 50 ml of DW and dilute ammonia solution with constant gentle stirring until the solution is just alkaline (to litmus paper). Allow the contents of the flask to stand for 30 minutes and then quantitatively filter through a NO. 4 sintered glass-crucible previously dried to a constant weight at 105°C. Wash the precipitate several times with DW, until the washings do not give a positive test for chloride (test with standard $AgNO_3$ Solution). Dry the residue to a constant weight at 105°C. Each gram of residue is equivalent to 1.306 g of $C_{20}H_{22}ON_3Cl$, 2HCl, $2H_2O$.

10.3.2.2.2. *Cognate Assays*

A few other pharmaceutical substances are also determined after conversion to free bases as recorded in Table : 10.4.

Table 10.4 : Substances Determined Gravimetrically by Conversion to Free Base

S.No.	Name of Substance	Qty. Prescribed	Drying Temp. (°C)	Calculations
1.	Phenacaine hydrochloride	0.5 g	105	Each g of residue ≡ 1.122 g of $C_{18}H_{22}N_2O_2$. HCl

10.3.2.3. Substances Assayed After Conversion to Free Compound

In certain specific cases either the pure pharmaceutical substance or dosage forms are quantitatively converted to free compound. This conversion to free compound is quantitative and hence forms the basis of gravimetric analysis. A few typical examples belonging to this category are, namely : progesterone suspension sterile, progesterone tablets, sodium lauryl sulphate, mephobarbital tablets and sorbitan monooleate.

10.3.2.3.1. *Mephobarbital Tablets*

Materials Required : Mephobarbital : 300 mg ; hexane : 100 ml ; chloroform : 150 ml ; alcohol (95% v/v) : l0 ml.

Procedure : Weigh and finely powder not less than 20 mephobarbital tablets. Transfer an accurately weighed portion of the powder equivalent to about 300 mg of mephobarbital to an extraction thimble. Extract with 15 ml of solvent hexane, allow the thimble to drain, transfer to a continuous extraction apparatus provided with a tared flask, and extract the mephobarbital with chloroform for 2 hours. Evaporate the chloroform on a steam bath with the aid of a current of air, cool, dissolve the residue in about 10 ml of alcohol, evaporate, dry the residue at 105°C for 1 hour, cool and weigh.

The weight of the residue represents the weight $C_{l3}H_{14}N_2O_3$ in the portion of the tablets taken.

10.3.2.4. Substances Assayed after Conversion to Derivatives or Substitution Products

In pharmaceutical drug analysis a host of organic pharmaceutical substances are invariably converted quantitatively to their corresponding derivatives by virtue of interactions with certain functional entities, namely : aldehyde, ketone, amino, carboxyl, phenolic, hydroxyl etc. However, in some cases it may be feasible to obtain uniform substitution products of organic pharmaceutical substances quantitatively, for instance : tetraido derivative of phenolphthalein is obtained from the phenolphthalein tablets. It is important to mention here that the number of organic pharmaceutical substances which may be analysed by this method is limited because of *two* vital reasons, they are :

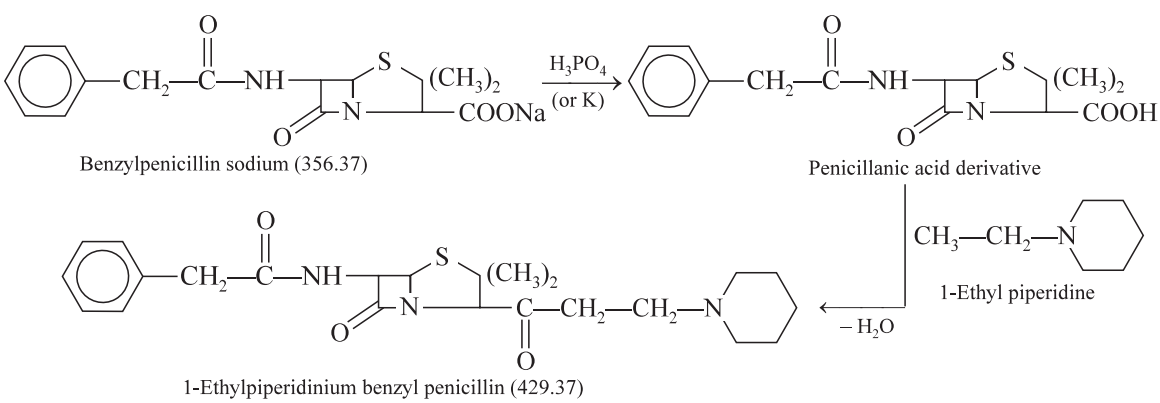
(*a*) the reversible nature of reactions, and

(*b*) the formation of products of side reactions simultaneously.

10.3.2.4.1. *Benzylpenicillin(Syn : Benzylpenicillin Sodium or Potassium Salt)*

Materials Required : Benzylpenicillin sodium (say) : 0.12 g ; amyl acetate (previously saturated with 1-ethylpiperidinium benzylpencillin at room temperature, cooled in ice and filtered) : 5.0 ml ; phosphoric acid (20% v/v) : 0.5 ml ; anhydrous sodium sulphate (freshly ignited and powdered) : 0.5 g ; dry acetone (previously saturated with 1-ethylpiperidinium benzylpenicillin at room temperature cooled in ice and filtered) : 3.0 ml ; 1-ethylpiperidine amyl acetate solution (prepared from l-ethyl piperidine, 1 .0 ml, and amyl acetate, 8.0 ml, saturated at room temperature with 1-ethylpiperidinium benzylpenicillin, cooled in ice and filtered) : 1.5 ml ; dry acetone in amyl acetate (1 : 1) previously saturated with 1-ethylpiperidinium benzylpenicillin : 2.0 ml ; solvent ether : 4.0 ml.

Theory : Benzylpenicillin (sodium or potassium salt) may be assayed gravimetrically by quantitative conversion to the 1-ethylpiperidinium benzylpenicillin derivative. The ultimate precipitation is caused by l-ethyl piperidine after the respective sodium or potassium salt of benzylpencillin has been duly converted with phosphoric acid to the corresponding penicillanic acid (*i.e.* parent acid) and the latter finally extracted with amyl alcohol. The reactions may be expressed as follows :



Therefore, we have :

$$C_{16}H_{17}N_2NaO_4S \equiv C_{23}H_{31}N_3O_3S$$

or $\quad 356.37 \text{ g } C_{16}H_{17}N_2NaO_4S \equiv 429.37 \text{ g } C_{23}H_{31}N_3O_3S$

or $\quad 0.8300 \text{ g } C_{16}H_{17}N_2NaO_4S \equiv 1 \text{ g of } C_{23}H_{31}N_3O_3S$

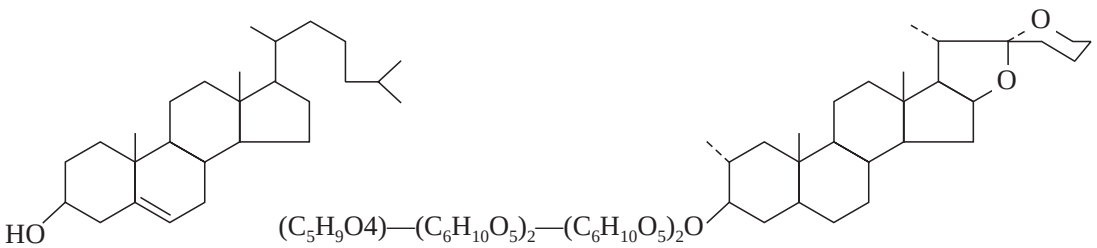
Procedure : Weigh accurately 0.12 g of benzyl penicillin sodium, dissolve in 5 ml of ice-cold DW in a flask and cool in an ice-bath. Add to it 5.0 ml of amyl acetate followed by 0.5 ml of ice-cold H_3PO_4, stopper, shake the contents immediately for 15 seconds, and centrifuge for 30 seconds. Remove the aqueous layer as completely as possible with the help of a pipette. Add 0.5 g anhydrous Na_2SO_4, stir the contents vigorously and cool in an ice-bath for 5 minutes. Centrifuge for about 30 seconds and again cool in ice-bath for 5 minutes. Pipette 3.0 ml of the supernatant liquid into a tared centrifuge tube. Add to it

3.0 ml of ice-cold acetone and 1.5 ml of 1-ethylpiperidine amyl acetate solution, stir, stopper the tube and cool in ice-bath for 2 hours. Now, centrifuge for 1 minute, break the surface with the help of a pointed glass rod, so that all crystalline particles are covered by liquid, and again centrifuge for 1 minute. Decant off the supernatant liquid, wash the precipitate with 2 ml of ice-cold dry acetone in amyl acetate (1 : 1) and again centrifuge for 1.5 minutes. Decant the supernatant liquid, wash twice with 2.0 ml portion of solvent ether, centrifuging for 1.5 minutes and decanting each time. Dry to constant weight under vacuum at room temperature. Each gram of residue is equivalent to 0.8300 g of $C_{16}H_{17}N_2NaO_4S$.

10.3.2.4.2. *Cholesterol*

Materials Required : Cholesterol : 0.1 g ; ethanol (90% v/v) : 12.0 ml ; digitonin solution (0.5% w/v in 90% v/v ethanol) : 40.0 ml ; ethanol (90% v/v) : 100 ml ; acetone ; carbon tetrachloride.

Theory : The assay of cholesterol is solely based on the fact that practically all 3 β-hydroxysterols *e.g.*, cholesterol, readily produces an insoluble molecular addition complex with pure digitonin (1 : 1)—a steroidal saponin isolated from either *Digitalis purpurea* or *Digitalis lanata.* The complex thus obtained is crystalline in nature, fairly stable and possesses very low solubilities.



The complexation of cholesterol and digitonin may be expressed as follows :

$$\underset{\text{Cholesterol}}{C_{27}H_{46}O} + \underset{\text{Digitonin}}{C_{56}H_{92}O_{29}} \longrightarrow \underset{\text{Digitonide Complex}}{C_{83}H_{138}O_{30}}$$

Therefore, 386.3 g $C_{27}H_{46}O \equiv 1616$ g $C_{83}H_{138}O_{30}$

or 0.2390 g $C_{27}H_{46}O \equiv 1$ g of $C_{83}H_{138}O_{30}$

Procedure : Weigh accurately about 0.1 g of cholesterol into a 100 ml flask and dissolve it in 12.0 ml ethanol. Insert the stopper and allow to stand at room temperature (25 ± 2°C) for 12 hours, filter through a Gooch crucible, and wash with 5.0 ml of ethanol. Mix the washings to the filtrate and add to it 40.0 ml solution of digitonin and make it warm to 60°C to ensure that the complexation is almost complete. Filter the precipitate of the resulting complex through a prepared Gooch crucible, previously dried to constant weight at 105°C. Wash the precipitate with ethanol followed by acetone and carbon tetrachloride, allow to drain as completely as possible, and dry to a constant weight at 105°C. Each g of the residue is equivalent to 0.2390 g of cholesterol.

Note : All solutions must be ice-cold.

10.3.2.4.3. *Thiamine Hydrochloride*

Materials Required : Thiamine hydrochloride : 0.5 g ; hydrochloric acid (≃ 11.5 N) : 2.0 ml ; silicotungstic acid solution (10% w/v in water) : 4.0 ml ; NO : 4-sintered glass-crucible ; dilute hydrochloric acid (1 part HCl + 19 parts H_2O) : 50 ml.

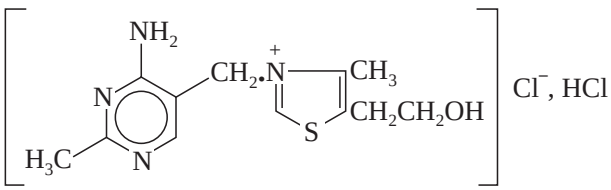
Theory : The gravimetric assay of thiamine hydrochloride is based upon the precipitation of it as thiamine silicotungstate with silicotungstic acid in a slightly acidic medium. It has been observed that the precipitating reagent is a complex silicate SiO_2, 12 WO_2, *n* H_2O having somewhat variable composition with regard to the degree of hydration. For a reasonably precise and accurate determination the precipitating reagent must contain ≮ 1.85% SiO_2 and ≮ 85% WO_3. Interestingly, the thiamine silicotungstate complex possesses more or less a constant composition.

The precipitation of insoluble thiamine silicotungstate may be designated by the following reaction :

$$2C_{12}H_{17}ON_4SCl, HCl + [SiO_2, 12WO_3] + 6H_2O \longrightarrow (C_{12}H_{17}ON_4SCl)_2, [SiO_2(OH)_2, 12WO_3], 4H_2O$$

or 674.6 $C_{12}H_{17}ON_4SCl, HCl \equiv$ 3480 g of Thiamine Silicotungstate

or 0.1958 g $C_{12}H_{17}ON_4SCl, HCl \equiv$ 1 g of Thiamine Silicotungstate



Thiamine Silicotungstate

Procedure : Weigh accurately 0.05 g of thiamine hydrochloride, previously dried at 105°C, and dissolve it in 50 ml DW in a 250 ml beaker having a stirring rod and watch glass cover. Add to it 2.0 ml of hydrochloric acid, heat to boiling and then add 4.0 ml of silicotungstic acid solution as rapidly as possible. Now, boil the solution gently for 2 minutes and quickly filter through a NO. 4 sintered-glass crucible, previously dried to a constant weight at 105°C. Wash the residue with a boiling mixture of HCl and H_2O (1 : 19) about 40 ml, then with DW 10.0 ml and ultimately with two portions of 5 ml each of acetone. Finally dry the residue to constant weight at 105°C. Each g of thiamine silicotungstate residue is equivalent to 0.1938 g of $C_{12}H_{17}ON_4SCl, HCl$.

Precautions :

(*a*) An excess of HCl is a must so as to produce a readily filterable precipitate,

(*b*) In case the sample is pure enough, the rate of addition of silicotungstic acid has little influence on the result, but on the contrary if the sample has significant impurity it may afford poor results,

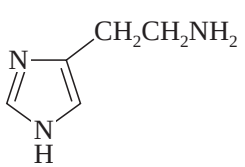
(*c*) To achieve complete complexation boiling must be done for more than 2 minutes, otherwise it would yield low results, and

(*d*) A 50-ml wash-liquid is quite ideal, further washings (volume) may offer poor results.

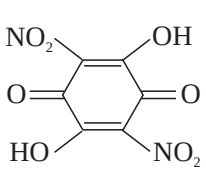
10.3.2.4.4. *Histamine Acid Phosphate ($C_5H_9N_3$, $2H_3PO_4$)*

Materials Required : Histamine : 0.15 g ; nitranilic acid solution (3.5% w/v in 95% ethanol) : 10.0 ml ; ethanol (95%) : 30.0 ml ; sintered-glass crucible (NO : 3) ; ether : 10.0 ml.

Theory : The gravimetric assay of histamine acid phosphate is based upon the formation of insoluble histamine-nitranilic acid complex as depicted in the following equation :



Histamine



Nitranilic acid

Therefore, we have :

$$\underset{\text{Histamine acid phosphate}}{C_5H_9N_3, 2H_3PO_4} + C_6H_2N_2O_8 \longrightarrow \underset{\text{Histamine-nitranilic acid complex}}{C_5H_9N_3.C_6H_2N_2O_8}\downarrow + 2H_3PO_4$$

or 307.1 g $C_5H_9N_3, 2H_3PO_4 \equiv$ 341.3 g $C_5H_9N_3.C_6H_2N_2O_8$

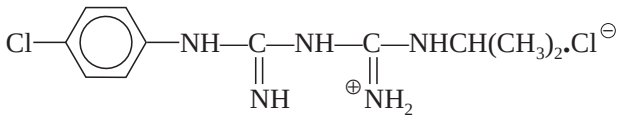
or 0.8998 g $C_5H_9N_3, 2H_3PO_4 \equiv$ 1 g of $C_5H_9N_3.C_6H_2N_2O_8$

Procedure : Weigh accurately about 0.15 g of histamine acid phosphate into a 250 ml beaker provided with a stirring rod and watch glass cover. Add to it 10.0 ml of DW to dissolve the sample. Now, add 10.0 ml of nitranilic acid solution, stir and allow to stand for 15 ininutes. Pour in 10.0 ml of ethanol, keep it in an ice-bath for 3 hours and filter through a No. 3 sintered-glass crucible, previously dried to a constant weight at 130°C. Transfer the precipitate quantitatively and wash it thoroughly with four quantities each of 5.0 ml of ethanol and ultimately with 10.0 ml of ether. Dry to constant weight at 130°C. Simultaneously, determine the loss in weight on drying a separate portion of the sample at 105°C. Each gram of the histamine-nitranilic acid complex is equivalent to 0.8998 g of $C_5H_9N_3$, 2 H_3PO_4.

10.3.2.4.5. *Proguanil Hydrochloride*

Materials Required : Proguanil hydrochloride : 0.6 g ; ammoniacal cupric chloride solution (dissolve 22.5 g of copper (II) chloride in 200 ml of DW and mix with 100 ml of 13.5 M ammonia) ; NO. 4 sintered-glass crucible ; mixture of dilute solution of ammonia and DW (1 : 5).

Theory : Gravimetric analysis of proguanil hydrochloride involves the precipitation of the proguanil-cupric complex that results on the addition of ammoniacal cupric chloride solution to a solution of proguanil hydrochloride. The reaction can be expressed by the following equation :



Proguanil hydrochloride

$$2C_{11}H_{l6}N_5Cl,\ HCl\ +\ CuCl_2 \xrightarrow{NH_3} (C_{11}H_{15}N_5Cl)_2Cu\ +\ 4\ HCl$$

Proguanil cupric complex

or 580.2 g $C_{11}H_{l6}N_5Cl$, HCl ≡ 568.9 g $(C_{11}H_{15}N_5Cl)_2Cu$

or 1.0199 g $C_{11}H_{l6}N_5Cl$, HCl ≡ 1 g of $(C_{11}H_{15}N_5Cl)_2Cu$

Procedure : Weigh accurately 0.6 g of proguanil hydrochloride into a 250 ml beaker fitted with a stirring rod and watch-glass cover. Add to it 50.0 ml of DW and heat gently to dissolve the sample. Chill the solution below 10°C in an ice-bath and then add ammoniacal-cupric-chloride solution with continuous stirring till the resulting solution attains a permanent deep-colour. Allow the solution to stand for 90 minutes to complete the complexation and then filter through a No. 4 sintered glass crucible previously dried to constant weight at 130°C. Transfer the precipitate quantitatively into the crucible, wash first with a mixture of dilute solution of ammonia and DW (1 : 5) adequately followed by cold water until the washings are practically colourless thereby showing the complete absence of soluble copper salts. Dry the precipitate to a constant weight at 130°C. Simultaneously, find out the loss in weight on drying with a separate portion of the sample at 105°C and incorporate this in the calculation. Each gram of proguanil-cupric-complex is equivalent to 1.0199 g of $C_{11}H_{l6}N_5Cl$, HCl.

10.3.2.4.6. *Benzethonium Chloride*

Theory : In general, quaternary nitrogen containing compounds like—choline chloride, acetylpyridinium chloride, benzethonium chloride, and bethanechol chloride readily form insoluble salts quantitatively with tetraphenyl boron and this puts forward the basis for the gravimetric assay of the above cited pharmaceutical substances.

The various reactions involved may be summarized and expressed as follows :

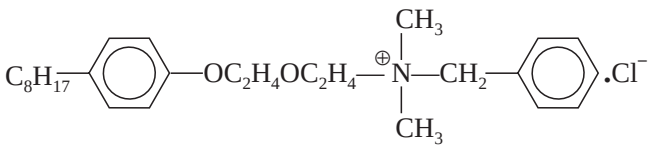
$$R_4N.Cl\ +\ Na(C_6H_5)_4B \longrightarrow R_4N(C_6H_5)_4B\downarrow\ +\ NaCl \qquad ...(a)$$

$$R_4N.Cl\ +\ Ind^- \longrightarrow R_4N.Ind\ +\ Cl^- \qquad ...(b)$$

$$R_4N.Ind\ +\ Na(C_6H_5)B \longrightarrow R_4N.(C_6H_5)_4B\ +\ Na^+\ +\ Ind^- \qquad ...(c)$$

Eq. (*a*) shows that the quaternary salt gets quantitatively precipitated by sodium tetraphenyl boron as the complexing agent. Eq. (*b*) depicts that quaternary compounds shall readily react with certain anionic dye, such as bromophenol blue, to yield a blue, chloroform-soluble complex.

Eq. (*c*) finally illustrates that the blue-coloured complex shall react quantitatively with sodium tetraphenyl boron to give an insoluble compound.



Bezethonium chloride

Therefore, we have :

$$C_{27}H_{41}O_2NCl + Na(C_6H_5)_4B \longrightarrow C_{27}H_{41}O_2N.(C_6H_5)_4B \downarrow + NaCl$$

or $C_{27}H_{41}O_2NCl \equiv C_{27}H_{41}O_2N.(C_6H_5)_4B$

or 446.45 g $C_{27}H_{41}O_2NCl \equiv$ 729.82 g $C_{27}H_{41}O_2N \cdot (C_6H_5)_4B$

or 0.6117 g $C_{27}H_{41}O_2NCl \equiv$ 1 g of $C_{27}H_{41}O_2N \cdot (C_6H_5)_4B$

Materials Required : Benzethonium chloride : 0.15 g ; Chloroform : 50 ml ; bromophenol blue solution (Dissolve with heating 0.2 g of bromophenol blue in 3 ml of 0.1 M NaOH and 10 ml of ethanol (96%). Allow to cool and dilute to 100 ml with ethanol 96%] : 50 ml ; sodium tetraphenyl borate solution (1% w/v in chloroform) : 50 ml ; sintered-glass crucible No : 4.

Procedure : Weigh accurately about 0.15 g of benzethonium chloride sample into a 250-ml beaker placed on a magnetic-stirrer and watch-glass cover. Add to it 25 ml of chloroform and warm gently to dissolve. Cool to ambient temperature and add suffcient bromophenol blue solution gradually till the solution yields a blue Chloroform-soluble complex. Now, add sodium tetraphenyl borate solution in small lots at intervals with constant stirring until the complete precipitation of insoluble benzethonium tetraphenyl borate complex takes place. Allow the solution to stand for 60 minutes to complete the complexation and subsequently filter through a No. 4 sintered-glass crucible previously dried to constant weight at 130°C. Transfer the precipitate quantitatively into the crucible and wash the precipitate with cold chloroform. Dry the precipitate to a constant weight at 110°C. Each gram of benzethonium tetraphenyl borate complex is equivalent to 0.6117 g of $C_{27}H_{41}O_2NCl$.

10.3.2.4.7. *Cognate Assays*

Quite a few official pharmaceutical substances and their respective dosage forms can be assayed gravimetrically after conversion to their corresponding derivatives or substitution products. Table 10.5 records some examples from *official compendia*.

Table 10.5 : Substances Assayed Gravimetrically by Conversion to Derivatives or Substitution Products

S.No.	Name of Substance	Qty. Prescribed	Drying Temp. (°C)	Calculations
1.	Piperazine citrate Tabs.	0.2 (≡ Piperazine Hydrate)	105	Each g of dipicrate residue ≡ 0.3568 g of $(C_4H_{10}N_2)_3$, $2C_6H_8O_7$
2.	Iodochlorhydroxyquin Tabs.	0.1	105	Each g of copper complex residue ≡ 0.9750 g of C_9H_5NOCl
3.	Phentolamine hydrochloride	0.5	105	Each g of trichloroacetate residue ≡ 0.7448 g of $C_{17}H_{19}N_3O$. HCl

THEORETICAL AND PRACTICAL EXERCISES

1. What are the advantages of '**gravimetric analysis**' over '**titrimetric analysis**' ? Give suitable examples to expatiate your answer.

2. How does the '**Law of Mass Action and Reversible Reactions**' help in accomplishing the gravimetric analysis ? Explain.

3. '**The principle of solubility product**' is the major factor in governing the gravimetric analysis. Justify the statement adequately with appropriate examples.

4. What is the critical role played by '**common ion effect**' in gravimetric analysis ? Explain the theoretical aspect and calculations involved in such an analysis.

5. How would you assay the following '**drugs**' gravimetrically :

(*i*) Sodium chloride (*ii*) Potassium alum

(*iii*) Barium sulphate (*iv*) Piperazine phosphate.

6. Gravimetric analysis may be accomplished by one of the following means and ways :

(*a*) Substances assayed after conversion to Free Acid,

(*b*) Substances assayed after conversion to Free Base,

(*c*) Substances assayed after conversion to Free Compound, and

(*d*) Substances assayed after conversion to Derivatives.

Give at least **ONE** suitable example from each method in support of your answer.

RECOMMENDED READINGS

1. Johnson, R.A., and J.D. O'Rourke, **J. Amer, Chem. Soc., 76**, 2124, 1954.

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5. Nielson, A.E., **Precipitates : Formation, Coprecipitation, and Aging,** In **Treatise on Analytical Chemistry,** ed. by I.M. Kolthoff and P.J. Elving, 2nd ed. Vol. 3, New York, Interscience Publishers, 1983.

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