PHARMACEUTICAL ANALYSIS

Theoretical Basis of Analysis: Complexometric Titrations

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Complexometric titrations, analysis of metals, chilometry, EDTA, ligands, chelate, masking & demasking agents, pM-indicators, hardness of water, analysis of calcium & magnesium.

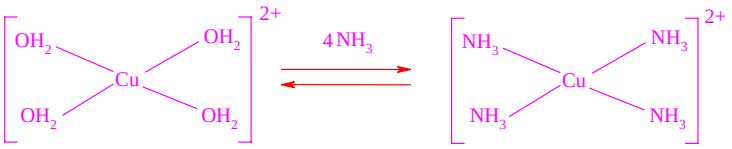
Introduction

The technique involves titrating metal ions with a complexing agent or chelating agent (Ligand) and is commonly referred to as complexometric titration. This method represents the analytical application of a complexation reaction. In this method, a simple ion is transformed into a complex ion and the equivalence point is determined by using metal indicators or electrometrically. Various other names such as **chilometric titrations**, chilometry, chilatometric titrations and EDTA titrations have been used to describe this method. All these terms refer to same analytical method and they have resulted from the use of EDTA (Ethylene diamine tetra acetic acid) and other chilons. These chilons react with metal ions to form a special type of complex known as **chelate**.

		Chilon		**Chelate**
Metal ion	+	Complexing agent	Metal-ion Indicators	Complex ion
(analyte; Cation)		Chelating agent Ligand Sequestering agent	→ pM Indicators	Metal co-ordination compound Metal complex Chelate compound

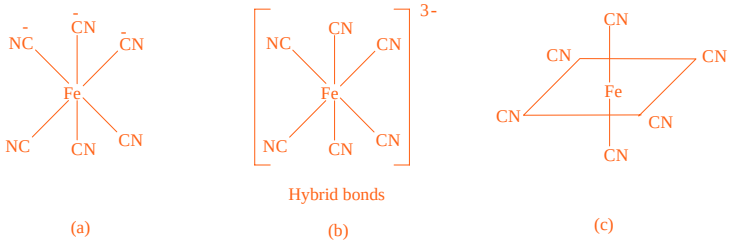
Metal ions in solution are always solvated, i.e. a definite number of solvent molecules (usually 2, 4 or 6) are firmly bound to the metal ion. However, these bound solvent molecules are replaced by other solvent molecules or ions during the formation of a metal complex or metal co-ordination compound.

The molecules or ions which displace the solvent molecules are called **Ligands**. Ligands or complexing agents or chelating agents can be any electron donating entity, which has the ability to bind to the metal ion and produce a complex ion. An example of a complexation reaction between Cu (II) ion and four ammonium molecules in an aqueous solution may be expressed by the following equation:



Bonding in Complexes

The bonds are either ordinary covalent bonds in which the metal and the ligand contribute one electron each, or co-ordinate bonds in which both electrons are contributed by the ligand. Thus, the hexacyanoferrate ion may be considered to consist of three ordinary covalent bonds and three co-ordinate bonds, although in the complex the bonds are identical hybrid bonds which have been shown to be directed towards the apices of a regular octahedron.



The hexacyanoferrate iron (III) ion

The negative charge on the complex ion is equal to the total number of the negative groups *minus* the valency of the metal ion. When neutral groups only are involved, the charge on the complex is positive and is equal to the metal ion, e.g. $[Cu(NH_3)_4]^{2+}$.

Werner's Co-ordination Number

Werner (1891) first noticed that for each atom there were an observed maximum number of small groups which can be accommodated around it. This number, which is called Werner's co-ordination number, depends purely upon steric factors and is in no way related to the valency of the ion. Thus, although the valency shell of the elements of the third period is theoretically capable of expanding up to 18 electrons, and that of the fourth to 32 electrons, there is, in practice, a limit to the number of small groups which can be accommodated owing to limitations of space around the ion. For example, in the $[BF_4]^-$ ion, the octet is completed and the maximum co-ordination number is reached, but in the $[AlF_6]^-$ ion the outer shell contains 12 electrons and cannot expand to the maximum number of 18 electrons since the maximum co-ordination number has been reached.

Within the limits imposed by Werner's co-ordination number, there is a tendency for the metal to attain or approach inert gas structure, and this is probably the driving force for complex formation.

Classification of Ligands

1. **Unidentate Ligands:** Ligands that are bound to metal ion only at one place are called **unidentate ligands (one toothed)**. NH_3, for example, is a unidentate ligand capable of complexing with cupric ions. Halide ions, cyanide ions and NH_3 are common examples of unidentate ligands. The formation of complex $Cu(NH_3)_4^{2+}$ proceeds in the following steps:

Step 1 $Cu^{2+} + NH_3 \rightleftharpoons Cu(NH_3)^{2+}$

Step 2 $Cu(NH_3)^{2+} + NH_3 \rightleftharpoons Cu(NH_3)_2^{2+}$

Step 3 $Cu(NH_3)_2^{2+} + NH_3 \rightleftharpoons Cu(NH_3)_3^{2+}$

Step 4 $Cu(NH_3)_3^{2+} + NH_3 \rightleftharpoons Cu(NH_3)_4^{2+}$

Considering the overall reaction:

$$Cu^{2+} + 4NH_3 \rightleftharpoons Cu(NH3)_4^{2+}$$

2. **Bidentate and Multidentate Ligands:** Many ligands are known that contain more than one group, capable of binding with metal ions. Such ligands are known as **multidentate** ligands or chelating agents. They include bidentate ligands (2 donar atoms), tridentate ligands (3 donar atoms), quadridentate ligands, etc.

Thus, ethylene diamine is an example of bidentate ligand. $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$

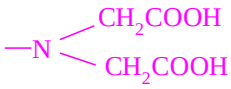
Ethylene diamine tetra acetic acid (EDTA) is an example of multidentate ligand.

Chelate Compound or Chelate

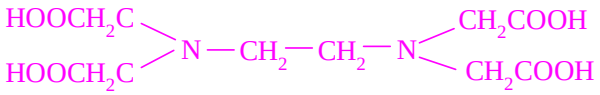
Complexes involving simple ligands, i.e., those forming only one bond are described as **co-ordination compound**. A complex of a metal ion with 2 or more groups on a multidentate ligand is called a **chelate** or a **chelate compound**. There is no fundamental difference between co-ordination compound and a chelate compound except that in a chelate compound, ring influence the stability of compound. Thus, a chelate can be described as a heterocyclic ring structure in which a metal atom is a member of ring. The stability of a chelate is usually much greater than that of corresponding unidentate metal complex.

Chelating agent

Ligands having more than one electron donating groups are called **chelating agents**. The most effective complexing agent in ligands are amino and carboxylate ions. All the multidentate ligands important in analytical chemistry contain the structure component as follows:

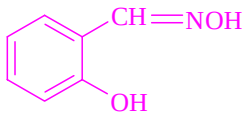


The solubility of metal chelates in water depends upon the presence of hydrophilic groups such as COOH, SO_3H, NH_2 and OH. When both acidic and basic groups are present, the complex will be soluble over a wide range of pH. When hydrophilic groups are absent, the solubilities of both the chelating agent and the metal chelate will be low, but they will be soluble in organic solvents. The term sequestering agent is generally applied to chelating agents that form water-soluble complexes with bi- or poly-valent metal ions. Thus, although the metals remain in solution, they fail to give normal ionic reactions. Ethylenediaminetetra-acetic acid is a typical sequestering agent, whereas, dimethylglyoxime and salicylaldoxime are chelating agents, forming insoluble complexes.



EDTA

Dimethylglyoxime



Salicylaldoxime

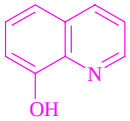
As a sequestering agent, ethylenediaminetetra-acetic acid reacts with most polyvalent metal ions to form water-soluble complexes which cannot be extracted from aqueous solutions with organic solvents. Dimethylglyoxime and salicylaldoxime form complexes which are insoluble in water, but soluble in organic solvents; for example, nickel dimethylglyoxime has a sufficiently low solubility in water to be used as a basis for gravimetric assay.

EDTA forms chelates with nearly all metal ions and this reaction is the basis for general analytical method for these ions by titration with a standard EDTA solution. Such titrations are called complexometric or chilometric or EDTA titrations.

Reagent EDTA

Disodium salt of EDTA is a water soluble chelating agent and is always preferred. It is non-hygroscopic and a very stable sequestering agent (Ligands which form water soluble chelates are called sequestering agents).

There are cheating agents that form water insoluble chelates with metal ions. E.g. - oxine or 8-hydroxy quinoline.



EDTA and 8-hydroxy quinoline are important reagents used in analytical chemistry. Sequestering agents are used to liberate or solubilize metal ions. The agents which form water insoluble chelates are used to remove the metal ions from solution by precipitation.

EDTA has the widest general application in analyses because of the following important properties:

- ☞ It has low price.
- ☞ The special structure of its anion which has 6 ligand atoms.
- ☞ It forms strainless five-membered rings.

Disodium EDTA is used as M/20 solution.

Purification of Disodium EDTA: Commercial samples of disodium EDTA may be purified for use as a primary standard by adding ethanol to a saturated aqueous solution until the first permanent precipitate appears; filter and add an equal volume of ethanol; filter the precipitated disodium EDTA, wash with acetone and ether, and dry to constant weight at 80°C, drying may require four days. The official material contains not less than 98% of the dihydrate.

Preparation of M/20 Disodium EDTA: Dissolve 18.6 gm of disodium EDTA in water and make the volume upto 1000 ml and standardize the prepared solution.

Standardization of Disodium EDTA: Weigh accurately about 200 mg of $CaCO_3$ in a titration flask. Add 50 ml of water and minimum quantity of dil. HCl to dissolve $CaCO_3$. Adjust the pH of the solution to 12 by adding NaOH. Add 300 mg of hydroxyl naphthol blue indicator and titrate with the prepared M/20 disodium EDTA solution, until the solution is deep blue in colour.

The HCl solubilizes the $CaCO_3$ by converting it to $CaCl_2$. The NaOH makes the solution alkaline and maintains the pH at about 12 so that the Ca-EDTA complex would be stable and any Mg, which might be present as a contaminant, would not react. The coloured Ca-indicator complex gives up Ca to EDTA, liberating the free uncomplexed indicator, which is blue.

Factors influencing EDTA reactions:

- ✶ The nature and activity of metal ion.
- ✶ The pH at which the titration is carried out.

- The presence of interfering ions such as CN^-, Citrate, Tartrate, F^- and other complex forming agents.
- Organic solvents also increase the stability of complex.

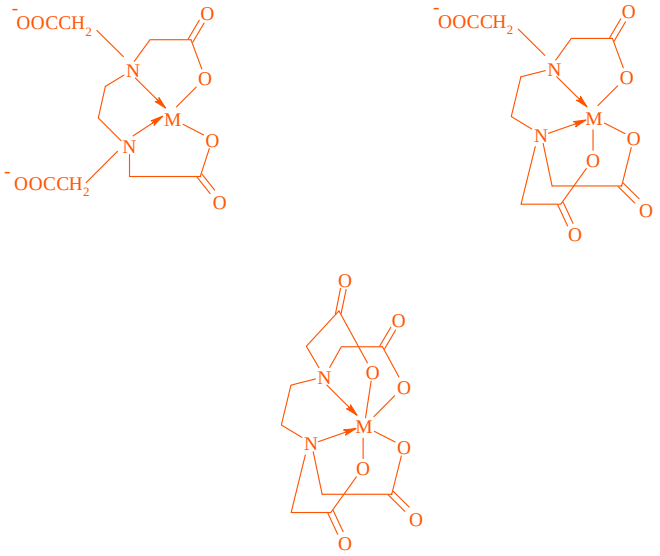
Nature and stability of metal complexes of Ethylenediaminetetra-acetic acid: Ethylenediaminetetra-acetic acid forms complexes with complexes with most cations in a 1:1 ratio, irrespective of the valency of the ion:

$$M^{2+} + [H_2X]^{2-} \rightleftharpoons [MX]^{2-} + 2H^+$$

$$M^{3+} + [H_2X]^{2-} \rightleftharpoons [MX]^{-} + 2H^+$$

$$M^{4+} + [H_2X]^{2-} \rightleftharpoons [MX] + 2H^+$$

where M is a metal and $[H_2X]^{2-}$ is the anion of the disodium salt (disodium EDTA) which is most frequently used. The structures of these complexes with di-, tri- and tetravalent metals contain three, four and five rings respectively:



Effect of pH on complex formation

Ethylenediamine tetra-acetic acid ionizes in four stages (pK_1=2.0, pK_2=2.67, pK_3=6.16 and pK_4=10.26) and, since the actual complexing species is Y^{4-}, complexes will form more efficiently and be more stable in alkaline solution. If, however, the solubility product of the metal hydroxide is low, it may be precipitated if the hydroxyl ion concentration is increased too much. On the other hand, at lower pH values when the concentration of Y^{4-} is lower, the stability constant of the complexes will not be so high. Complexes of most divalent metals are stable in ammonical solution. Those of the alkaline earth metals, such as copper, lead and nickel, are stable down to pH 3 and hence can be titrated selectively in the presence of alkaline earth metals. Trivalent metal complexes are usually still more firmly bound and stable in strongly acid solutions; for example, the cobalt(III) edetate complex is stable in concentrated hydrochloric acid. Although most complexes are stable over a fair range of pH, solutions are usually buffered

at a pH at which the complex is stable and at which the colour change of the indicator is most distinct.

Colour of complexes: There is always a change in the absorption spectrum when complexes are formed and this forms the basis of many colorimetric assays.

Stability of Complexes : The general equation for the formation of a 1:1 chelate complex, MX, is

$$M + X \rightleftharpoons MX$$

where M is the metal ion and X the chelating agent.

$$\therefore \text{ Stability Constant (K)} = [MX]/[M][X]$$

where [ ] represents activities. Increase in temperature causes a slight increase in the ionization of the complex and a slight lowering of K. the presence of electrolytes having no ion in common with the complex decreases K, whilst the presence of ethanol increases K, probably due to the suppression of ionization.

Principle of Complexometric Titration

Many principles of acid-base titrations are used in complexometric titration. In complexometric titration, the free metal ions disappear as they are changed into complex ions. In acid-base titrations, the end point is marked by sudden change in pH. Similarly, in EDTA titration, if we plot pM (negative log of metal ion concentration) v/s volume of titrant, we will find that at the end point, the pM rapidly increases (Fig. 1). This sudden pM raise results from removal of traces of metal ions from solution by EDTA.

Any method, which can determine this disappearance of free metal ions, can be used to detect end point in complexometric titrations. End point can be detected usually with an indicator or instrumentally by potentiometric or conductometric (electrometric) method.

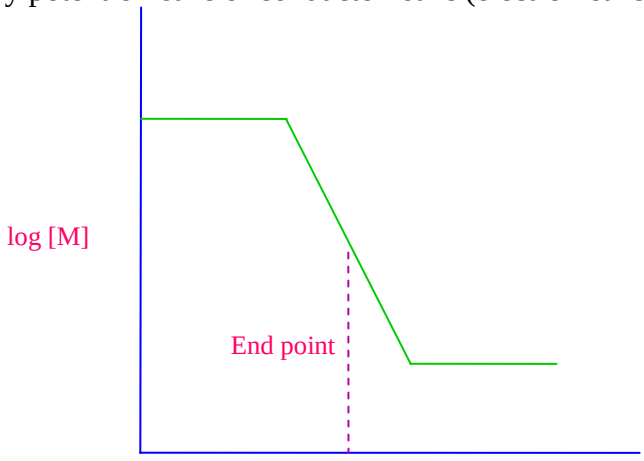


Fig. 1

There are three factors that are important in determining the magnitude of break in titration curve at end point.

1. **The stability of complex formed:** The greater the stability constant for complex formed, larger the charge in free metal concentration (pM) at equivalent point and more clear would be the end point.

2. **The number of steps involved in complex formation:** Fewer the number of steps required in the formation of complex, greater would be the break in titration curve at equivalent point and clear would be the end point.

3. **Effect of pH:** During a complexometric titration, the pH must be constant by use of a buffer solution. Control of pH is important since the H^+ ion plays an important role in chelation. Most ligands are basic and bind to H^+ ions throughout a wide range of pH. Some of these H^+ ions are frequently displaced from the ligands (chelating agents) by the metal during chelate formation.

   Equation below shows complexation between metal ion and H^+ ion for ligand:

   Thus, stability of metal complex is pH dependent. Lower the pH of the solution, lesser would be the stability of complex (because more H^+ ions are available to compete with the metal ions for ligand). Only metals that form very stable complexes can be titrated in acidic solution, and metals forming weak complexes can only be effectively titrated in alkaline solution.

Methods of End Point Detection

End point in complexometric titration can be detected by the following two methods:

1. **Indicators:** The end point in complexometric titrations is shown by means of pM indicators. The concept of pM arises as follows:

If K is the stability constant,

$$K = [MX]/[M][X]$$

then,
$$[M] = [MX]/[X]K$$

or
$$\log [M] = \log [MX]/[X] - \log K$$

and
$$pM = \log [X]/[MX] - pK$$

Therefore, if a solution is made such that [X] = [MX], pM = -pK (or pM = pK', where K' = dissociation constant). This means that, in a solution containing equal activities of metal complex and free chelating agent, the concentration of metal ions will remain roughly constant and will be buffered in the same way as hydrogen ions in a pH buffer. Since, however, chelating

agents are also bases; equilibrium in a metal-buffer solution is often greatly affected by a change in pH. In general, for chelating agents of the amino acid type (e.g., edetic acid and ammonia triacetic acid), it may be said that when [X] = [MX], pM increases with pH until about pH 10, when it attains a constant value. This pH is, therefore, usually chosen for carrying out titrations of metals with chelating agents in buffered solutions.

The pM indicator is a dye which is capable of acting as a chelating agent to give a dye-metal complex. The latter is different in colour from the dye itself and also has a low stability constant than the chelate-metal complex. The colour of the solution, therefore, remains that of the dye complex until the end point, when an equivalent amount of sodium EDTA has been added. As soon as there is the slightest excess of EDTA, the metal-dye complex decomposes to produce free dye; this is accomplished by a change in colour.

Over 200 organic compounds form colored chelates with ions in a pM range that is unique to the cation and the dye selected. To be useful, the dye-metal chelates usually will be visible at 10^{-6}-10^{-7} M concentration. Many of these indicators also have the typical properties of acid-base indicators and the colour changes are the result of the displacement of the H^+ by a metal ion. Metal indicators must comply with the following requirements-

- Compound must be chemically stable throughout the titration.
- It should form 1:1 complex which must be weaker than the metal chelate complex.
- Colour of the indicator and the metal complexed indicator must be sufficiently different.
- Colour reaction should be selective for the metal being titrated.
- The indicator should not compete with the EDTA.

Mechanism of action of indicator: Let the metal be denoted by M, indicator by I and chelate by EDTA. At the onset of the titration, the reaction medium contains the metal-indicator complex (MI) and excess of metal ion. When EDTA titrant is added to the system, a competitive reaction takes place between the free metal ions and EDTA. Since the metal-indicator complex (MI) is weaker than the metal-EDTA chelate, the EDTA which is being added during the course of the titration is chelating the free metal ions in solution at the expense of the MI complex. Finally, at the end point, EDTA removes the last traces of the metal from the indicator and the indicator changes from its complexed colour to its metal free colour. The overall reaction is given by:

$$\underset{\text{(Colour of metal-indicator complex)}}{MI} + M + EDTA \longrightarrow M\text{-}EDTA + \underset{\text{(original color of indicator)}}{I}$$

Structures of some important indicators used in complexometric titrations are given in Fig. 2. Many compounds have been used as indicators (Table-1), like:

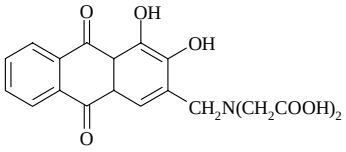
- Triphenyl methane dyes
- Phthalein and substituted phthaleins
- Azo dyes
- Phenolic compounds

Table-1: Indicators used in complexometric titrations

S.No.	Name of the Indicator	Colour change	pH range	Metals detected
1.	Mordant black II	Red to Blue	6-7	Ca, Ba, Mg, Zn, Cd, Mn, Pb, Hg
	Eriochrome blackT			
	Solochrome blackT			
2.	Murexide or Ammonium purpurate	Violet to Blue	12	Ca, Cu, Co
3.	Catechol-violet	Violet to Red	8-10	Mn, Mg, Fe, Co, Pb
4.	Methyl Blue	Blue to Yellow	4-5	Pb, Zn, Cd, Hg
	Thymol Blue	Blue to Grey	10-12	
5.	Alizarin	Red to Yellow	4.3	Pb, Zn, Co, Mg, Cu
6.	Sodium Alizarin sulphonate	Blue to Red	4	Al, Thorium
7.	Xylenol range	Lemon to Yellow	1-3	Bi, Thorium
			4-5	Pb, Zn
			5-6	Cd, Hg

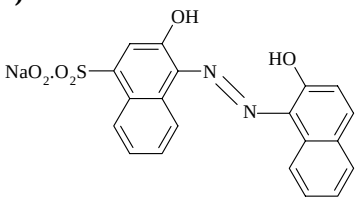
Fig. 2: Structures of some pM indicators

1)



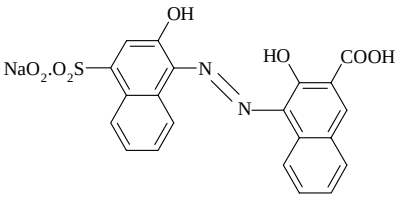
Alizarin fluorine blue (alizarin complexone)

2)



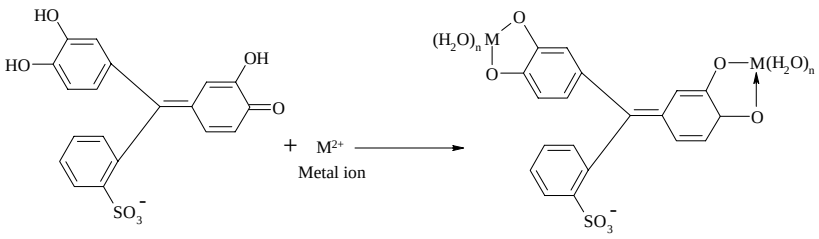
Calcone (mordant black 17)

3)



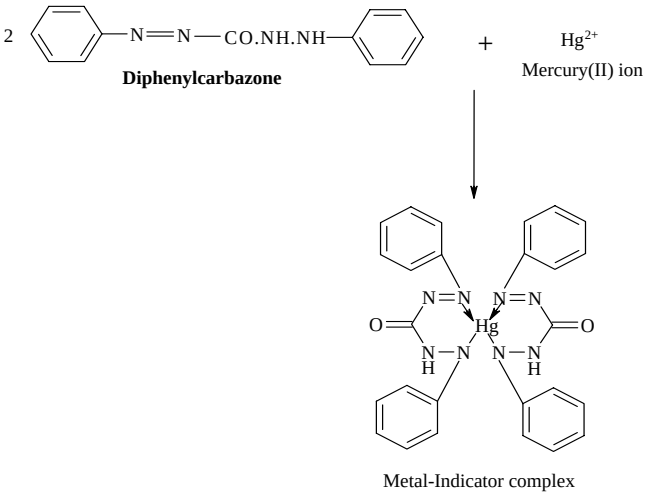
Calcon carboxylic acid

4)



Catechol violet Metal-indicator complex

5)



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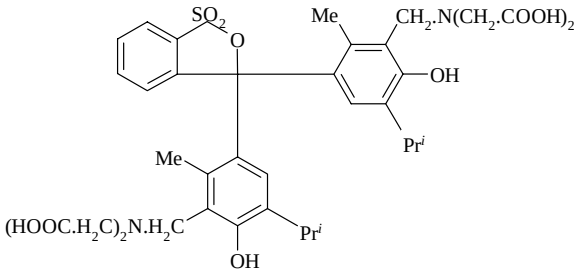

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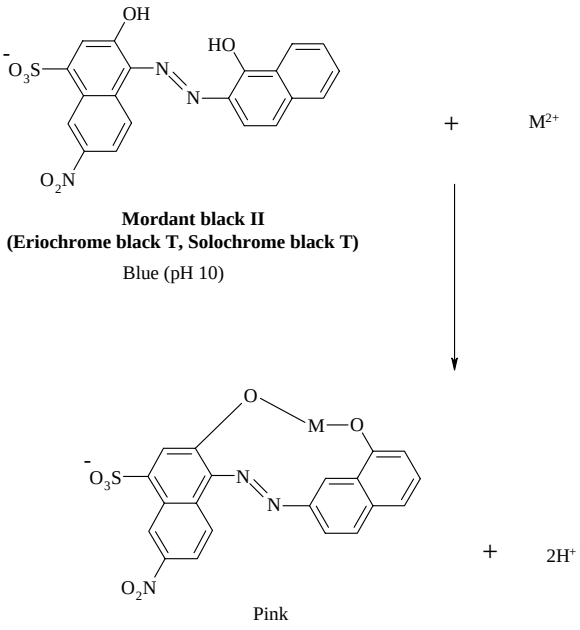

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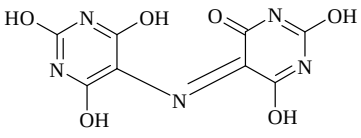
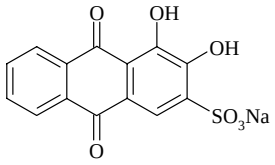


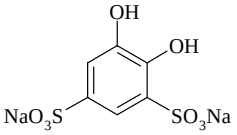
Murexide (violet at pH 10)

9)

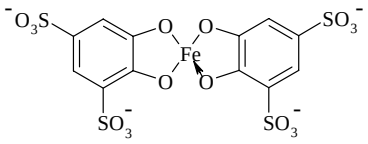


Sodium Alizarine sulphonate

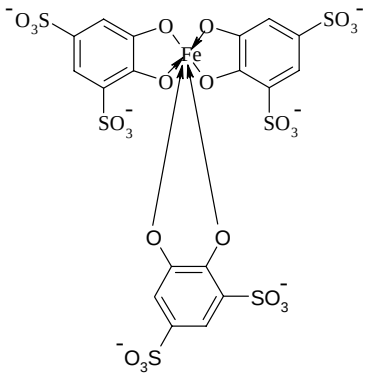
10)



Tiron
(disodium,1,2-dihydroxyphenol-3,5-disulphonate)

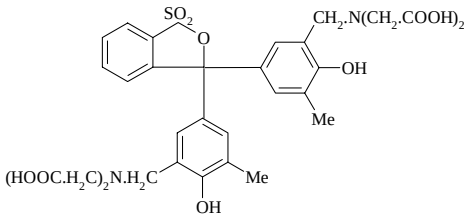


Blue complex

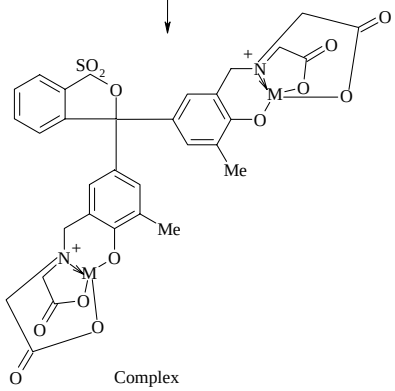


Red complex

11)



Xylenol Orange



Complex

2. Instrumental methods of End point detection:

- ✓ **Spectrophotometric detection:** The change in absorption spectrum when a metal ion of a complexing agent is converted to the metal complex, or when one complex is converted to another can usually be detected more accurately and in more dilute solution by spectrophotometric than by visual methods. Thus, in disodium EDTA titrations an accurate end point can be obtained using 0.001M solutions. In practice an indicator giving a colour change in the visible region is generally employed, but coloured ions may be titrated without an indicator using spectrophotometric methods. Also it is sometimes possible to use an end point in the ultraviolet region for ions and complexes which are colourless in the visible region.

- ✓ **Amperometric titration:** The effect of complex formation on the half-wave potential of an ion is to render it more negative. If the electrode potential is adjusted to a value between that of the half-wave potential of the free cation and that of the complex, and disodium EDTA solution is added slowly, the diffusion current will fall steadily until it equals the residual current, that is, until the last trace of free cation has been complexed. This is the end point and the amount of standard disodium EDTA solution added is equivalent to the amount of metal present.

- ✓ **Potentiometric titration:** Since disodium EDTA reacts preferentially with the higher valency state of an ion, it will reduce the redox potential according to the equation,

$$E = E_0 + \log_e [Ox]/[Red]$$

where, E = the potential of the electrode

E_0 = the standard electrode potential

[Ox] = activity of the ions in the oxidized state

[Red] = activity of the ions in the reduced state

This method is of limited application owing to the lack of suitable indicator electrodes. Iron(III) and copper(II), however can be titrated in this way. Back titration of excess disodium EDTA with ferric chloride in acid solution is possible for some ions.

- ✓ **High frequency titrator:** This method is particularly suitable for dilute solutions, in some cases with concentrations as low as 0.0002M. The ions may be titrated directly in buffered solution or excess reagent can be added to the unbuffered solution and the liberated protons titrated with standard alkali. Since buffer solution and other extraneous electrolytes reduce the sensitivity of the titration, their concentration must be kept to a minimum.

Types of Complexometric Titrations

Complexometric titrations are of 4 types:

1. **Direct Titration:** It is the simplest and the most convenient method used in chelometry. In this method, the standard chelon solution is added to the metal ion solution until the end point is detected. This method is analogous to simple acid-base titrations. E.g.-calcium gluconate injection, calcium lactate tablets and compound sodium lactate injection for the assay of calcium chloride ($CaCl_2.6H_2O$).

Limitations -slow complexation reaction
-Interference due to presence of other ions

2. **Back Titration:** In this method, excess of a standard EDTA solution is added to the metal solution, which is to be analyzed, and the excess is back titrated with a standard solution of a second metal ion. E.g. - Determination of Mn. This metal cannot be directly titrated with EDTA because of precipitation of $Mn(OH)_2$. An excess of known volume of EDTA is added to an acidic solution of Mn salt and then ammonia buffer is used to adjust the pH to 10 and the excess EDTA remaining after chelation, is back titrated with a standard Zn solution kept in burette using Eriochrome blackT as indicator. This method is analogous to back titration method in acidimetry. e.g.- ZnO

3. **Replacement Titration:** In this method the metal, which is to be analyzed, displaces quantitatively the metal from the complex. When direct or back titrations do not give sharp end points, the metal may be determined by the displacement of an equivalent amount of Mg or Zn from a less stable EDTA complex.

$$Mn^{+2} + Mg\ EDTA^{-2} \longrightarrow Mg^{+2} + Mn\ EDTA^{-2}$$

Mn displaces Mg from Mn EDTA solution. The freed Mg metal is then directly titrated with a standard EDTA solution. In this method, excess quantity of Mg EDTA chelate is added to Mn solution. Mn quantitatively displaces Mg from Mg EDTA chelate. This displacement takes place because Mn forms a more stable complex with EDTA. By this method Ca, Pb, Hg may be determined using Eriochrome blackT indicator.

4. **Indirect Titration:** This is also known as Alkalimetric titration. It is used for the determination of ions such as anions, which do not react with EDTA chelate. Protons from disodium EDTA are displaced by a heavy metal and titrated with sodium alkali.

$$M^{n+} + H_2X^{-2} \longrightarrow MX^{(n-4)} + 2H^{+}$$

e.g. - Barbiturates do not react with EDTA but are quantitatively precipitated from alkaline solution by mercuric ions as 1:1 complex.

Method: Barbiturate to be analyzed is taken in a flask and heated with excess of mercury in alkaline solution. When precipitated Hg-barbiturate complex is formed, it is filtered and dissolved in excess of standard EDTA solution. The unreacted EDTA solution is then back titrated with a standard Zn solution.

Some important elements which could be determined by complexometric titration are as follows:

i) **Direct Titration** : Analysis of Cu, Mn, Ca, Ba, Br, Zn, Cd, Hg, Al, Thallium, Sn, Pb, Bi, Vanadium, Cr, Mo, Gallium, Fe, Co, Ni, and Pd.

ii) **Indirect Titration:** Analysis of Na, K, Ag, Au, As, C, N, P, S, Cl, Br, I and F.

Titration Selectivity, Masking and Demasking Agents

EDTA is a very unselective reagent because it complexes with numerous doubly, triply and quadruply charged cations. When a solution containing two cations which complex with EDTA is titrated without the addition of a complex-forming indicator, and if a titration error of 0.1% is permissible, then the ratio of the stability constants of the EDTA complexes of the two metals M and N must be such that $K_M/K_N \geq 10^6$ if N is not to interfere with the titration of M. strictly, of course, the constants K_M and K_N considered in the above expression should be the apparent stability constants of the complexes. If the complex-forming indicators are used, then for a similar titration error $K_M/K_N \geq 10^8$.

The following procedures will help to increase the selectivity:

- Use of masking and demasking agents
- pH control.
- Use of selective metal indicators.
- Classical separation
- Solvent extraction
- Removal of anions
- Kinetic masking

➢ Use of masking and demasking agents:

Masking agents act either by precipitation or by formation of complexes more stable than the interfering ion-EDTA complex.

a) **Masking by Precipitation:** Many heavy metals e.g.- Co, Cu and Pb, can be separated either in the form of insoluble sulphides using Sodium sulphide, or as insoluble complexes using thioacetamide. These are filtered, decomposed and titrated with disodium EDTA. Other common precipitating agents are sulphate for Pb and Ba, oxalate for Ca and Pb, fluoride for Ca, Mg and Pb, ferrocyanide for Zn and Cu, and 8-hydroxy quinoline for many heavy metals. Thioglycerol ($CH_2SH.CHOH.CH_2OH$) is used to mask Cu by precipitation in the assay of lotions containing Cu and Zn.

b) Masking by Complex formation: Masking agents form more stable complexes with the interfering metal ions. The most important aspect is that the masking agent must not form complexes with the metal ion under analysis. The different masking agents used are enlisted below:

☞ **Ammonium fluoride** will mask aluminium, iron and titanium by complex formation.

☞ **Ascorbic acid** is a convenient reducing agent for iron(III) which is then masked by complexing as the very stable hexacyanoferrate(II) complex. This latter is more stable and less intensely coloured than the hexacyanoferrate(III) complex.

☞ **Dimercaprol (2,3-Dimercaptopropanol); ($CH_2SH.CHSH.CH_2OH$).** Cations of mercury, cadmium, zinc, arsenic, tin, lead and bismuth react with dimercaprol in weakly acidic solution to form precipitates which are soluble in alkaline solution.

All these complexes are stronger than the corresponding edetate complexes and are almost colourless. Cobalt, copper and nickel form intense yellowish-green complexes with the reagent under the above conditions. Cobalt and copper, but not nickel, are displaced from their edetate complexes by dimercaprol.

☞ **Potassium cyanide** reacts with silver, copper, mercury, iron, zinc, cadmium, cobalt and nickel ions to form complexes in alkaline solution which are more stable than the corresponding edetate complexes, so that other ions, such as lead, magnesium, manganese and the alkaline earth metals can be determined in their presence. Of the metals in the first group mentioned, zinc and cadmium can be demasked from their cyanide complexes by aldehydes, such as formaldehyde or chloral hydrate (due to the preferential formation of a cyanohydrin), and selectively titrated.

☞ **Potassium iodide** is used to mask the mercury(II) ion as $(HgI_4)^{2-}$ and is specific for mercury. It can be used in the assay of mercury(II) chloride.

☞ **Tiron (disodium catechol-3,5-disulphonate)** will mask aluminium and titanium as colourless complexes. Iron forms highly coloured complexes and is best masked as its hexacyanoferrate(II) complex.

☞ **Triethanolamine $[N(CH_2.CH_2.OH)_3]$** forms a colourless complex with aluminium, a yellow complex with iron(III), the colour of which is almost discharged by adding sodium hydroxide solution, and a green manganese(III) complex which oxidizes mordant black II. For these reasons, if murexide is used in the presence of iron and manganese, it is best to amsk them with triethanolamine; similarly, mordant black II can be used in the presence of triethanolamine-aluminium complex.

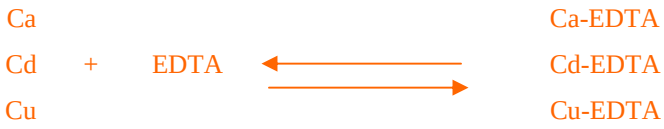
Demasking: It is the process in which the masked substance regains its ability to enter into a particular reaction. This enables to determine a series of metal ions in one solution containing many cations.

Example of using masking and demasking agents in complexometry is the analysis of 3 metals, Cu, Cd and Ca. the following method of analysis is followed:

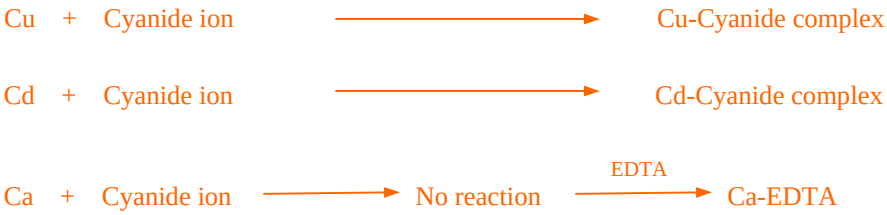
1. Direct titration of the mixture with the EDTA gives the sum of the 3 metals.

2. Cu and Cd may be masked with the addition of cyanide to the solution, leaving only Ca ion.
3. When formaldehyde or chloral hydrate is added to the cyanide containing mixture, only Cd is demasked and the EDTA titrates the sum of Ca and Cd. In this manner, the concentration of three ions is determined by 3 individual titrations.

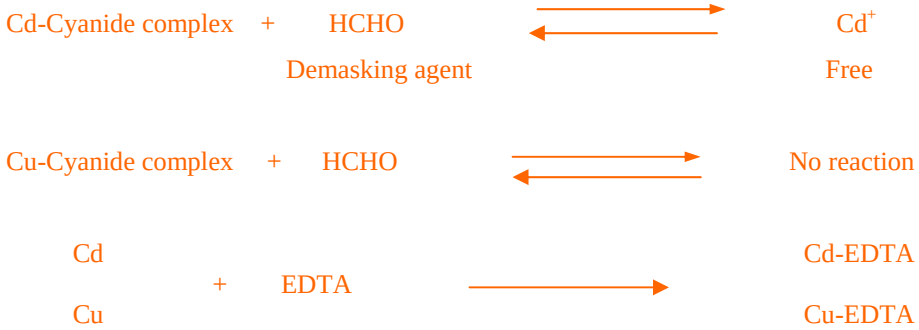
Step 1. All three metals are titrated.



Step 2. Only Ca is titrated.



Step 3. Cd and Ca are titrated.



- **pH control Method:** The formation of a metal chelate is dependent on the pH of the reaction medium. In weakly acid solution, the chelates of many metals are completely dissociated such as alkaline earth metals, whereas chelates of Bi, Fe^{3+} or Cr are readily formed at this pH. Thus, in acidic solution, Bi can be effectively titratedwith a chelating agent in the presence of alkaline earth metals. This method is based upon the differences in stability of the chelates formed between the metal ions and the chelating agent.

- **Use of selective metal indicators:** These indicators are the metal complexing agents which react with different metal ions under various conditions. Several selective metal indicators have been used and they are specific for a particular ion.

- **Classical separation:** These may be applied if they are not tedious; thus the following precipitates may not be used for separations in which, after being re-dissolved, the cations can be determined complexometrically: CaC_2O_4, nickel dimethylglyoximate, $Mg(NH_4)PO_4$, $6H_2O$, and CuSCN.

- **Solvent extraction:** This is occasionally of value. Thus, Zinc can be separated from copper and lead by adding excess of ammonium thiocyanate solution and extracting the resulting zinc thiocyanate with 4-methylpentan-2-one (isobutyl methyl ketone); the extract is diluted with water and the zinc content determined with EDTA solution.

- **Removal of Anions:** Anions, such as orthophosphate, which can interfere in complexometric titrations, may be removed using ion exchange resins.

- **Kinetic masking:** This is a special case in which a metal ion does not effectively enter into the complexation reaction because of its kinetic inertness. Thus the slow reaction of chromium (III) with EDTA makes it possible to titrate other metal ions which react rapidly, without interference from Cr (III); this is illustrated by the determination of iron (III) and chromium (III) in a mixture.

Applications of Complexometric Titrations

Complexometric titrations have been employed with success for determination of various metals like Ca, Mg, Pb, Zn, Al, Fe, Mn, Cr etc. in different formulations that are official in I.P., and also for the determination of Hardness of water.

Determination of Calcium in different formulations: Calcium can be determined in almost every formulation by EDTA-titrations. e.g.- Five membered heterocyclic rings are formed with EDTA, which are stain-free, and thus highly stable.

Ca-EDTA Complex

- **Assay of $CaCO_3$:** Accurately weighed amount of $CaCO_3$ is dissolved in water and then acidified with HCl. A mixture of naphthol green and murexide is then added and titrated with EDTA, kept in burette.

  1ml of M/20 disodium EDTA ≡ 0.005005 gm of $CaCO_3$

- **Calcium Lactate tablets:** 20 tablets are finely powdered and an accurately weighed amount of the powder, representing about 0.5gm of calcium lactate, is transferred to a crucible, ignited until free from carbon and then cooled. 10 ml water is added and the residue is dissolved by adding dropwise dil. HCl solution.this solution is then transferred to a container, diluted to 150 ml with water and the assay is completed as is given under general procedure.

  1ml of M/20 disod. EDTA ≡ 0.01542gm of Ca lactate

- **Calcium Lactate injection:** Measure out a suitable volume of the injection, equivalent to about 0.5gm of Ca lactate. Transfer to the titration flask and proceed as given under general procedure.

- **Calcium Gluconate:** An accurately weighed quantity (0.8gm) is dissolved in water (150ml) containing dil HCl (5ml). to the acidified solution is added, solution of NaOH (15ml), murexide indicator (4mg), solution of naphthol green (3ml). The reaction mixture is titrated with M/20 disod. EDTA until the solution is deep blue in colour.

1ml of M/20 disod. EDTA ≡ 0.02242gm of Ca gluconate

- **Calcium Gluconate injection:** An accurately measured volume of the injection, equivalent to 0.8gm of Calcium gluconate is taken in a titration flask and proceeded as above.

- **Calcium Gluconate tablet:** 20 Tablets are finely powdered. An accurately weighed amount of the powder, equivalent to 0.8gm of Calcium gluconate is transferred to a crucible and proceeded as described under Calcium lactate tablets.

Determination of Magnesium: Dissolve an accurately weighed sample (75mg) of Mg in sufficient water to make 100ml. pipette out 50ml of this solution in a titration flask, add 50ml water, 5ml of NH_3 buffer solution and a few drops of eriochrome blackT as indicator. Titrate it to a deep blue colour.

Each ml of M/20 disodium EDTA ≡ 0.02432 gm of Mg

This method could be used for the assay of Mg stearate and Mg sulphate.

Determination of Hardness of Water: Water hardness due to Ca and Mg is expressed as the amount of Ca and Mg ions in ppm. Actually, the hardness is due to both Ca and Mg salts but he two are determined together in the titration. The total Ca and Mg is titrated with standard EDTA solution using eriochrome blackT as indicator.

Method: Disodium salt of EDTA has the general formula: $Na_2H_2Y_2.H_2O$, where Y is the tetravalent anion of EDTA. When Ca is titrated with H_2Y^{2-}, a very stable complex is formed.

$$Ca^{2+} + H_2Y^{2-} \rightleftharpoons CaY^{2-} + 2H^+$$

Mg^{2+} forms a similar complex which is far less stable than the Ca complex. When a sample containing Ca and Mg ions is titrated with a solution of EDTA, the Ca^{2+} are first complexed as CaY^{2-}. As more reagent is added, all the Ca^{2+} is combined as complex. Mg ion forms MgY^{2-}. The desired end point if the titration is the point at which all the Ca and Mg ions of the solution have combined with the complexing agent.

Suggested Reading:

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5. A.H. Beckett and J.B. Stenlake: Practical Pharmaceutical Chemistry, Vol I & II. The Athlone Press of the University of London.
6. www.pubmed.com