1.1. General introduction

Transition metal ion chelate complexes\(^1\) are exploited by industry in the large-scale purification of amino acids and in the synthesis of wide range of drugs and drug precursors containing an amino carboxylic acid moiety. Chiral ligand exchange chromatography, which utilizes stereoselective binding to an immobilized chiral ligand (selector), is commonly used in industry for the separation of racemic mixtures of amino acids and their derivatives. Newer technologies for scalable continuous separation of chiral racemates that present the selector either directly in solution or on the surfaces of stable micelles. These industrial applications are often best carried out at temperatures far from ambient or physiological conditions. Efficient design and optimization of these technologies therefore requires knowledge of chemical equilibria\(^2-4\) within the system and its dependence on temperature\(^5\). Complexes as the term is usually used in inorganic chemistry, include compounds composed of a metal atom or ion and one or more ligands (atoms, ions, or molecules) that formally donate electrons to the metal\(^6\). The complexes\(^7\) are the compounds containing coordinate bonds between electron pair donor as the ligand and electron pair acceptors as the metal atoms or ions. The number of electron pairs donated to the metal is known as its coordination number and thereby many complexes exist which exhibit coordination number of two, four or six. In order for a pair of electrons to be donated from ligand to a metal ion, there must be an empty (d) orbital on the metal ion to accept the pair of electrons. In a complex\(^8\), a central atom or ion is coordinated by one or more molecules or ions (ligands) which act as Lewis bases, forming coordinate bonds with the central atom or ion; the latter acts as a Lewis acid. Atoms in the ligands that are directly bonded to the central atom or ions with donor atoms\(^9\). In chemical language, it is known as acid base\(^10\) coordination complex or coordination compound, and any substance can accept a pair of electron is called as Lewis acids whereas any substance donate a pair of electrons commonly called as Lewis bases. When a ligand contains two or more donor
atoms close to each other, the metal complex formed is said to be a chelate\textsuperscript{11-12}, and the process is referred as chelation. The chelating ring may be ionic or covalent depending on the nature of ligand. Thus, the formation\textsuperscript{13} of mixed-ligand complexes is very important to understanding the behaviour of metal ligand complexes which are made up of a central metal ion and ligands in addition to the solvent molecules required to make up the coordination sphere of the metal ion. Such metal ion ligand complexes are quite common in biological and analytical systems. Thus, an understanding of the significance of metal ions in biological systems\textsuperscript{14} may unravel the mysteries surrounding the protein-substrate interactions and the control mechanisms that determine the coordination and coordination tendency of the metal ions bound at the active sites of many enzymes in enzyme-metal ion-substrate reactions, considering the high affinity of ion for donor atoms like oxygen and nitrogen etc\textsuperscript{15-16}. Motivated mainly by the desire of understanding metal ion-biomolecular interactions, in the past 25 years, there has been an increased interest in the study of mixed-Chelate complexes in solution. Several effects have been established, as responsible for the stability enhancement of such complexes compared to statistical expectations. A great number of biologically relevant metal ion-ligand interactions involve the formation of hexacoordinated complexes for which tris-chelates represent a very important fraction\textsuperscript{17}. Mixed ligand complexes derived from transition metal ions and designed ligands having specific functional groups are useful in biomimetic studies for exploring the role of such metal ions in enzymatic processes\textsuperscript{18}. Metal ions in biological system\textsuperscript{19} play key roles in the structural organization and activation of certain enzymes, which are involved in the transfer of genetic information from DNA, leading to the synthesis of specific proteins. It is well known that ternary complexes play an important role in biological processes, as exemplify by many instances in which enzymes are known to be activated by metal ions. Ternary complexes\textsuperscript{20} have also been implicated in the storage and transport of active substances through biological
membranes. Much attention has been paid recently to the study of ternary complexes of transition metals with molecules of biological and pharmaceutical interest. Furthermore, it has been suggested that the presence of metal ions in biological fluids, could have a significant effect on the therapeutic action of drugs. Amino acids and their metal complexes are equally important compounds; they have frequent utilization in both biological and chemical applications. Ternary complexes formed between metal ions and two different types of biological ligands, namely heteroaromatic nitrogen bases and amino acids (or peptides) may be considered as models for substrate metal ion–enzyme interactions and other metal ion mediated biochemical interactions. Among these compounds, metal complexes are known to play a significant role either in naturally occurring biological systems or as pharmacological agents, such as antitumour, anticandida, antimycobacterial, antimicrobial activity, etc. In a number of biochemical processes, metal ion is involved in mixed ligand complex formation and ligand catalyzed complex formation reactions. The history of complexes and the interpretation of complexes begin with Alfred Werner (1866-1919). Complexes were known much earlier; many complexes have been used as pigments but with the gradual development of analytical methods. The formulas of most of compounds became known late in 19th century and theories of structure and bonding in complexes become possible. A special class of higher order complexes was the historically important cobalt and platinum ammine complexes. These complexes formed the archetypes of Werner, in which he achieved his theory (coordination theory); Werner called them coordination compounds, consisting of a central metal atom and a certain number of coordinated molecules or ions. Coordination compounds are of great practical importance. Coordinating agents are used in metal-ion sequestration or removal, solvent extraction, dyeing, leather tanning, electroplating, catalysis, water softening, and in other industrial processes. For example, vitamin B$_{12}$ is a coordination compound of cobalt, the hemoglobin of our blood is a coordination compound of iron, the haemocyanin of
invertebrate animal blood is a coordination compound of copper, and the chlorophyll of green plants is a coordination compound of magnesium.

1.2. Importance of stability constants

The stability constant of complexes has been found to be greater than zero, is perhaps one of the most convincing pieces of evidence for the existence of the complex species ML\textsubscript{n} in solution. Moreover, if all the possible stability constants for a given system have been determined, it is possible, in principle, to calculate the equilibrium concentration\textsuperscript{33-36} or activity of each of the species present under a known set of experimental conditions. Such exact knowledge of the composition of a solution is essential for a correct interpretation of its optical and kinetic properties of partition equilibria and of its biological behavior. The importance of mixed ligand complexes\textsuperscript{37} in nature is evidence since a great deal of biological reactions occurs within the coordination sphere of metal ion complexes. These species has an important biological implication because of enhanced probability of bringing stability constants of the mixed ligand complex, species therefore be well known tools for solution chemists, biochemists and chemist in general to help to determine the properties of metal ligand reactions in aqueous medium of biological relevance. The equilibrium constant K can also be utilized to evaluate the different thermodynamic parameters\textsuperscript{38-41} like free energy Δ\textit{G}, enthalpy Δ\textit{H}, entropy Δ\textit{S}. Complex formation is most favored by the negative enthalpy and positive entropy changes as may be expressed by the equation:

\[
\log K = \frac{\Delta S - \Delta H/T}{2.303R}
\]  

(1)

Though significant advances have been made in the field of coordination chemistry, there are still large numbers of mixed ligand complexes whose structures, reactivities, and applications are not known. One of the successful methods to investigate such problems is the study of equilibria involving the formation of mixed metal chelates\textsuperscript{42-43} in solution. It is, therefore, considered appropriate to deal with some important aspects like the simultaneous and/or
stepwise formation of mixed ligand complexes in aqueous and aquo-organic media.

1.3. Factors affecting the stability of metal complexes

There are many factors which assist the formation of a complex and sometimes hidden factors are working against the same. The capacity of a metal ion to form a complex with a ligand is mainly decided by its environment, which decides the stability of the complex. Some of the factors which affect the stability of the complexes is discussed below.

1.3.1. Solvent

The ability of the ligand to displace the solvent molecule is dependent on how weakly the metal-ion is solvated; also the extent of association of the ion pairs i.e. metal ion and its anion is dependent on the dielectric constant of the medium. The solubility of organic ligands and complexes being low in aqueous medium, it becomes necessary to select an organic medium in which the compound under study has a higher solubility.

1.3.2. Temperature

The effect of temperature on the stabilities of metal is described by the van’t Hoff equation. Knowledge of stability at one temperature is enough to arrive at free energy change of the reaction from the relation:

\[ \Delta G^0 = -2.303 \text{RT} \log K \]

However, to estimate the changes in enthalpy (\(\Delta H^0\)) and entropy (\(\Delta S^0\)) of the reaction, variation in stabilities of complexes between known temperatures are required as is evident from the following relations:

\[ \Delta H^0 = 2.303R \frac{T_1}{T_2} \left( (\log K_1 - \log K_2)/\left(1 - T_2/T_1\right) \right) \]

Where \(\log K_1\) and \(\log K_2\) are the stability constants of the simple binary complexes at temperature \(T_1\) and \(T_2\) respectively. So, the complex formation reaction is carried out at different temperatures. Since the temperature, is one of the parameter, which influences the power of complexation. If the complex formation is an exothermic process i.e. \(\Delta H\) is negative, an increase in temperature will
enhance the stability of the complex, and if it is an endothermic process i.e. $\Delta H$ is positive, an increase in temperature will reduce the stability of the complex.

1.3.3. Effect of metal ions

The stability constant of complexes depends upon the nature of metal ions\textsuperscript{51-55}. Wahid U. Malik\textsuperscript{65} reported that the stabilities of high spin complexes of the ions between Mn(II) and Zn(II) with a given ligand frequently vary in the order: Mn(II)<Fe(II)<Co(II)<Ni(II)<Cu(II)>Zn(II). This order is called natural order (Irving-Williams order of stability), the stability of metal complexes increases with increasing atomic number up to the end of transition series and then decreases to zinc. The metal ligand bond strength depends on the metal to a considerable extent though it is attached to oxygen, nitrogen or sulphur containing ligands and the increase in bond strength may be due to the electrostatic force of attraction between metal ions and the ligand. Stability\textsuperscript{56} was seen to increase with electronegativity, ionization potential, and atomic number of the metal.

1.3.4. Influence of ionic strength

The change in ionic strength\textsuperscript{57-58} changes the activity coefficients of the species. In all physico-chemical/ electroanalytical methods an excess amount of supporting electrolyte is used in order to maintain the ionic strength, which keeps the activity coefficients of the reactants or products constant. The variations in the stabilities constants with ionic strength are used to calculate the thermodynamic stabilities and to establish the nature of the interactions. This fact is of great importance in biological fluids where parameters such as ionic strength and dielectric constant are extremely variable, depending on the charge over the species. The stability may increase or decrease with ionic strength. If the binary and ternary complexes carry equal charges, the stability increases with ionic strength i.e. when aqueous medium activity decreases.
1.3.5. Nature of the ligand

The effect the nature of ligand on the stability of complex depends on prime acid group, donor atom with a lone pair of electron, chelate effect and size of chelate ring etc.

**Prime acid group**

In Prime acid group, that the metal displaces the acidic hydrogen and takes its place. The bond formed is usually a covalent sigma (σ) bond.

**Donor atom with lone pair of electrons**

In donor atom with lone pair of electrons, that the donor atom shares its lone pair of electrons forming a coordinate bond. Basicity of the ligand is often considered as important factor in deciding the stability of the complex. Higher the donor capacity of the ligand, the most of basic, it is a stronger complex forming. The basicity largely depends on how easily the ligand can donate pair of electron; it depends on its electronegativity and the type of bonding that has it, with its neighboring atoms. For example, if a lone pair of electron occupy a hybridized orbital having more of ‘p’ character, it will comparatively be loosely held and hence the donating ability i.e. basicity will be higher. Thus, order will be sp< sp²< sp³. Complexes, in which π-acceptor ligands are present, still have higher stability. This happens when the ligand has vacant orbital for the acceptance of electrons from the metal ions. 2, 2-Dipyridyl though less basic than ethylenediamine is stronger coordinating agent with transition metal ions and this enhanced stability due to the possibility of π- bond formation. Presence of resonance in the ligand especially at the reacting site enhances the stability of the complex.

**Chelate effect**

One of the most striking properties of the chelate ring compounds is their unusual stability. Complexes of acetyl acetone with many metals have remarkable stability. This is in sharp contrast to the low stability of coordination compounds containing simple ketone. This enhanced stability was termed ‘Chelate effect’ by
Schwarzenbach. The formation of multiple fused rings around the central metal atom confers an even greater stability than the formation of single ring. For example Cu (II) ethylenediamine-bis-acetyl acetone which contains three interlocked rings can be heated to redness without undergoing decompositions.

**The size of the chelates ring**

The stereochemistry of metal chelates ring differs from that of carbon ring system in the sense that all the atoms in the chelate ring are not of the same size and some of the bond angles normally vary from $109^0$ or $120^0$ as a result of the directed valences of metal ion. The chelate effect varies with the size of the ring, the most favoured being the five and six member ring chelates.

**1.3.6. Steric factor within the complex**

Clashing groups of two coordinated ligands result in the distortion of bond angles and a decrease in the stability constant. A steric effect, affects the mode of packing of ligands around a central metal ion, and imposes a particular geometric arrangement like planar, tetrahedral or octahedral to the complex.

**1.3.7. Effect of substituent**

Substitution of a group in a chelating agent may affect the stability of the metal chelates by influencing the basicity of the donor atom or may interfere with enhance the resonance of the chelating ring.

**1.3.8. Nature of the donor atom**

Generally oxygen, nitrogen and sulphur are most common donor atoms, which give large ligand field splitting and form the more stable complexes with metal ions which are particularly sensitive to ligand field stabilization. While donor atoms that produce small ligand field tend to form relatively less stable complexes with cations which are insensitive to ligand field stabilization.

**1.4. Theories of coordination compounds**

**1.4.1. Alfred Werner**

The work of French-born Alfred Werner i.e. Werner theory was abrupt break with the classical theories of valence and structure, Werner postulated two
types of valences, primary or ionizable valence (inorganic) and secondary or non-ionizable valence (non-inorganic). Every metal in an oxidation state also has a definite coordination number, whereas primary valence can be satisfied only by anions, secondary valence can be satisfied not only by anion but also by neutral molecules. The secondary valences are directed in space around the central metal atom, to clarify ideas of chemical bonding.

1.4.2. Valence bond theory

Valence bond theory was proposed by Linus Pauling and others\(^6^7\) in the 1930. This theory was simple that correlate the types of hybridization with the geometry of the complexes. According to this theory, complexes are form due to the interaction of Lewis bases (ligands) and Lewis acid (metal) with the formation of coordinate covalent type bond between them. It fails to explain some spectroscopic and magnetic properties of transition metal complexes.

1.4.3. Crystal field theory (CFT)

This theory was first proposed by Hans Bethe and modified by J.H.VanVleck\(^6^8\). It considered bonding between the metal ion and ligand due to a purely electrostatic attraction. It was successfull in interpreting many important properties of complexes. CFT treat the ligand atoms as ionic ligand or neutral ligand and thereby, if the ligand is ionic approaches to the central metal ion by negative charge, if the ligand is neutral molecules, it approaches to central metal ion by negative poles; thereby the central metal ion is surrounded by dipoles. CFT failed to explain complexes formation between metal ion involving S and P-orbitals and ligand containing \(\pi\)-orbital and it also failed to interpret the strengths of ligands.

1.4.4. Molecular orbital theory (MOT)

According to this theory, the metal –ligand \(\sigma\)-bonding in complexes results from overlap of suitable atomic orbitals of the central metallic cation with ligand \(\sigma\)-orbitals, the metal-ligand \(\sigma\) bond formation in the complexes take place due to overlap of orbital along with the axis. The \(\sigma\)-orbitals of ligand have been
represented as $\sigma_x, \sigma_y, \sigma_z, \sigma_{-x}, \sigma_{-y}, \sigma_{-z}$, these $\sigma$-orbitals, in order to form metal ligand $\sigma$-bonds, overlaps more effectively with only those valence atomic orbitals of metal ions, which are having their lobes along the axes. Such atomic orbitals are $4s, 4p_x, 4p_y, 4p_z, 3d_x$ and $3d_{-y}^2$ and $3d_{-z}^2$, since these orbitals have their lobes lying along the axes. The remaining three atomic orbitals namely $3d_{x^2-y^2}, 3d_{yz}$ and $3d_{zx}$ non-bonding orbitals. These can overlap sidewise with filled or unfilled $\pi$-orbitals of the same ligand to form metal ligand $\pi$-bonds. Metal ligand complexes$^{67-68}$ results from the overlap of atomic orbitals of central metallic cation with the orbitals of ligand and the bonding of complex between metal ion and ligand occurs when the condition of energy and symmetry and overlap permit.

1.5. Literature survey

The work of coordination chemistry was first begins in the twentieth century. It was mostly related to stepwise formation of complexes$^{69}$. The equilibrium constant involving the formation of a metal complex from the aquo metal ion and the most basic form of the ligand$^{70}$ is a standard measure of the effectiveness of the ligand in coordinating metal ions. Most complex formation reactions are measured in aqueous medium under controlled conditions of the ligands which are not soluble in water but are soluble in organic solvents and the stability constant with metal ions are often determined in mixed solvents. Potentiometric measurement was first used for the measurement of stability constants by Arrhenius, Ostwald and Nernst$^{71}$, who provided the basis for the introduction of electrodes resonance reversibly and selectively to only one species present in solution. The potential of electrodes provide sufficient information for the determination of stability constants of complex formation reactions. The investigation of Bjerrum, Bronsted and McGuinnesson activity coefficients and the development of theory of strong electrolytes in solution by Debye and Huckel in 1923, formed the basis for exact studies of metal ions and anions in solution. The determination of empirical formulas and overall formation constants were pioneered by workers such as Von Euler and Bodlander. Stepwise formation of
complexes was first demonstrated for the system \( \text{Hg}^{2+} \), \( \text{Cl}^- \) by Abegg and coworkers\(^72\). Also Bodlander and Grossman\(^73\) were the first to apply the idea of an ionic medium to control the ionic strength of solution. The stepwise hydrolysis constants of \( \text{Cr}^{3+} \) were described by Bjerrum\(^74\) that described the thiocyanate complexes of \( \text{Cr}^{3+} \) and calculated the six-step stability constants. The introduction of general methods for computing stepwise stability constants was developed by J.Bjerrum, the J.Bjerrum used a large excess of the ligand (ammonia) to prevent hydrolysis and developed approximation methods for the calculation of the metal ammonia stability constant of a number of complexes. In 1945 a classic method by Calvin and Wilson appeared in which the stability constant of a number of complexes were calculated without Bjerrum simplifications by the use of exact algebraic treatment of equilibrium constants and mass balance equations. A large part of the work was carried out by three research groups, those of Sillen\(^75-76\), Bjerrum's group and Schwarzenbach and coworkers Subsequently a group involving Martell\(^77\) and coworkers, the extensive treatment of the determination of stability constants by Rossotti and Rossotti. Other major works that should be mentioned by Bailer and his students in which many reaction mechanisms is described and more modern text by Lewis and Wilkins\(^78\) in which the use of ligand field theory.

1.6. Importance of metal ion in biological system

Metal ions play a vital role in biological processes; metal ion concentration\(^79\) must be maintained within proper ranges in the biological fluids. If the concentration of a given essential metal ion is too low, processes, which need to use the same ion, will be adversely affected and the organism can suffer from metal ion deficiency. Once the concentration of a given metal ion is above a lower threshold, there will be enough of that ion to complete biological functions. However, due to industrial uses of some of these metals\(^80\), some people can be exposed to too much higher concentrations as a result of which they suffer from many serious diseases. Furthermore, diseases release metals into the blood stream.
The concentration of these metals in blood and urine of human beings can be reduced by ligand therapy. A lot of ligands have been used as antidote to combat metal poisoning. Transition metals are non-essential heavy metals that are normally present in very low concentration in our environment.

1.6.1. Cobalt

Cobalt is the essential metal for many organisms including mammals. The activity of cobalt is confined to functions of vitamin B$_{12}$ and enzyme. The use of vitamin B$_{12}$ in biology required in archaebacteria and this association of cobalt with early anaerobic organisms. Cobalt used as catalysts to handle compounds such as CH$_4$, H$_2$, H$_2$S in atmosphere; Cobalt is toxic moderately when injected intravenously to mammals.

1.6.2. Nickel

Nickel is a rare metal in biology. The activity of nickel is confined to one enzyme, urease, which acts in redox processes, although the symbiotic anaerobic bacteria still use nickel in some dihydrogan reaction. Free anaerobic bacteria, especially methanogens, have also kept the nickel hydrogenase and other nickel enzymes, but the methanogens belong to the special class of bacteria. Nickel is toxic and a dangerous to the health of humans from nickel poisoning is essential. In many microorganisms its transport is highly regulated by the cell.

1.6.3. Copper

Copper is present in a large number of enzymes, many involved in electron transfer, activation of oxygen and other small molecules such as oxides of nitrogen, methane and carbon monoxide, superoxide dismutation and even invertebrates, oxygen transport, the copper binding protein in serum that plays an important role in iron metabolism, and by the terminal oxidase of the mitochondrial respiratory chain. Cytochrome oxidase which requires both haem iron and copper for its activity. Copper levels are maintained at extremely low levels by a series of copper chaperone proteins in mammals.
1.6.4. Zinc

Zinc is an essential element for the normal functioning of most of living organisms and its deficiency can lead to reduction of normal growth. It is a major regulatory ion in the metabolism of cells, the feel for this role comes from gross biological considerations though their molecular nature begins to appear in zinc fingers and in the link to amino acid, nucleotide and haem syntheses. Today it is difficult to know where all the zinc inside cellular systems is located. Zinc\textsuperscript{84} is found to be associated with DNA and RNA. It is very important in the activity of many enzymes, bacteria and is toxic in excess.

1.7. Classification of drugs

Drugs are the compounds, which interact with a biological system to produce a biological response, pharmacology embrace\textsuperscript{85}. The knowledge of the history, that the physical and chemical properties, biochemical and physiological effect, mechanism of action and other uses of drugs are very important. The goal of drug therapy is to prevent, cure, or control various disease states. To achieve this goal, adequate drug doses must be delivered to the target tissues so that therapeutic yet nontoxic levels are obtained. According to their therapeutic action, the drugs may be classified\textsuperscript{86-88} into the following types:

1. Drugs acting on the central nervous systems: There are some groups of drugs acting on the central nervous systems such as:
   i. Analgesics are a group of drugs which are used to relieve pain in the body. They selectively depress central nervous system and thus make the body insensible to pain without the loss of consciousness; for example aspirin, ibuprofen, and diclofenac sodium.
   ii. Epilepsy drugs: Epilepsy is a physical condition that occurs when there is a sudden, change in the brain activity and the drugs are used as antiepileptic such as gabapentin, barbiturates, benzodiazepines, carbamazepine, divalproex, ethosuximide, lamotrigine.
iii. Anxiolytic and hypnotic drugs: Anxiety is an unpleasant state of tension, apprehension, or uneasiness a fear that seems to arise from a sometimes unknown source. Alprazolam, chlordiazepoxide, clonazepam and clorazepate are some example of this class.

2. Drugs stimulating the peripheral nervous: theses drugs act by altering the transmission of impulses between synapses or between neuroeffector junctions. The drugs are divided into groups according to the type of neuron involved in their mechanism of action such as cholinergic and adrenergic drugs.

3. Drugs acting on the cardiovascular hematopoietic and renal systems: This group includes drugs that affect cardiovascular function as well as those that act on blood vessels and the renal system. These include:
   i. Antiarrhythmic drugs: The drugs which are used for the modification of cardiac rate and rhythm such as quinine, quinidine, precainamide, lidocaine, propranolol and isoproterenol.
   ii. Antihypertensive drugs: Any agent used for reducing blood pressure and its accompanying symptoms are called antihypertensive drug e.g. atenolol, hydralazine, minoxidil, diazoxide, prazosin, bretylium tosylate, lidocaine.
   iii. Vasodilators: These represent a group of drugs which act primarily on the vascular system. Their therapeutic effect is due to their ability to dilate coronary vessels and is therefore used for treating coronary artery diseases and especially in angina pectoris. Drugs are used such as amyl nitrite, glyceryl trinitrate.

4. Chemotherapeutic drugs: chemotherapeutic drugs are used in the treatment of infectious diseases. These diseases are caused by certain species of metazoan, protozoa, fungi, bacteria, ricketissa and viruses. Drugs active on theses pathogenic agents may be further divided into the following types:
i. Anthelmintic agents: A type of drug or herbal preparation given to destroy parasitic worms or expel them from the body. There three major groups of helminths such as nematodes, trematode and cestodes infect humans.
   a. Drugs are used for treatment of nematodes such as diethyl carbamazine, ivermectin, mebendazole and thiabendazole.
   b. Drugs are used for treatment of trematode such as praziquantal.
   c. Drugs are used for treatment of cestodes such as niclosamide, Praziquantel and albendazole.

ii. Antimalarial agents: It is the drug of choice in the treatment of erythrocytic and the drugs are used for treatment of malaria such as chloroquine, hydroxychloroquine.

iii. Antiprotozoal drugs: protozoal diseases such as malaria, amebiasis, leishmaniasis, trypansomiasis, trichomoniasis and drugs are metronidazole, furazolidone, and hydroxychloroquine.

iv. Antiseptic agents: It is a substance which prevents the growth of micro-organisms as long as it remains in contact with them whereas a disinfectant is one which kills the organisms outright, the ideal antiseptic would destroy bacteria, spores, fungi, viruses, and other infective agents without harming the tissues. The drugs are used for treatment of septic infective are fluorinated quinolones like norfloxacin, ciprofloxacin. HCl.

v. Antibacterial agents: Any drug that destroys bacteria or inhibits their growth e.g. ceftriaxone sodium.

vi. Antifungal agents: Infectious diseases caused by fungi are called mycoses, and they are often chronic in nature. The fungal infections that is most difficult to treat the systemic mycoses, which are often life-threatening and drugs that use as antifungal such as benzoic acid, salicylic acid, salicylanide.
vii. Antibiotics agents: It is a chemical substance produced by or derived from living cells which is capable, in small concentration, to inhibit the life processes or even destroying the micro-organisms e.g. chloramphenicol, dactinomycin, doxorubicin and daunorubicin.

viii. Antiviral agents: viruses are obligate intracellular parasites; few drugs are selective enough to prevent viral replication without injury to the host for example idoxuridine, methisazole and amantadine.

ix. Anticancer agents: Cancer chemotherapy strives to cause a lethal cytotoxic event or apoptosis in the cancer cell that can arrest a tumors progression. The drugs which are used as anticancer include docetaxal, paclitaxel, vinblastine, vinoristine and vinorelbine.

x. Antacids agents: The consumption of certain food or indigestion of food causes an increase in the acidity of the gastric juice. This condition is generally described as hyper acidity. It causes irritation and inflammation of gastric lining and if left untreated causes gastric ulcers. The drugs are used as antiulcer such as pantoprazole sodium, omeprazole, esmeprazole and rebeprazole.

xi. Anti-inflammatory agents: Inflammation may be defined as series of changes that take place in the living tissues following injury. The drugs which are used as anti-inflammatory includes ibuprofen, mefenamic acid and naproxen.

1.8. Determination of stability constant of binary complexes

The stability constant of complex $^{89-90}$ in the solution is usually determined by the knowledge of measurement of equilibrium constants (K) for complex forming reaction $^{91}$. The knowledge of stability constant, therefore, is of immense help to rationalize our understanding the behaviors of metal chelate in the solution.

1.8.1. Bjerrum method

The use of Bjerrum technique $^{92-97}$ is to determine the stability constant of metal-complexes from the concentration of metal, free ligand and total ligand
concentration. The equilibrium constant of the free ligand is a prerequisite to the occurrence of the following equilibria:

\[
H_2L^+ \rightleftharpoons K_1 HL + H^+ \quad K_1 = \frac{[H^+][HL]}{[H_2L^+]} \quad (2)
\]

\[
HL \rightleftharpoons K_2 L^- + H^+ \quad K_2 = \frac{[H^+][L^-]}{[HL]} \quad (3)
\]

Where \(H_2L^+, \ HL\) and \(L^-\) are the diprotonated, monoprotonated and the ligand anion respectively. The values of \(pK_1\) and \(pK_2\) were determined according to the equations: \(pK_1 = -\log K_1\) and \(pK_2 = -\log K_2\) and to calculate the concentration of \(H_2L^+, \ HL\) and \(L^-\) which are present in the reaction medium, most previous studies consider that the complexation processes were carried out by one of these species. This study takes into account that all of these species could act as a ligating species. Due to the presence of positive charge of the metal ions, an expected repulsion between \(H_2L^+\) and metal ions may occur, resulting that, the diprotic species \(H_2L^+\) were excluded as a ligating species. In this case, the most probable ligating species are \(HL\) and/or \(L^-\), the stiochiometric stability constants of the possible suggested reactions; the deprotonated ligand anion \(L^-\) could acts as a ligating species according to the following equilibria:

\[
M + L^- \rightleftharpoons ML \quad \beta_1^{[L^-]} = \frac{[ML]}{[M][L^-]} \quad (4)
\]

\[
M + 2L^- \rightleftharpoons ML_2 \quad \beta_2^{[L^-]} = \frac{[ML_2]}{[M][L^-]^2} \quad (5)
\]

The \(\beta_1^{[L^-]}\) and \(\beta_2^{[L^-]}\) are the overall stability constants of the complexes formed from the reaction between ligand and metal ion. From equations 1, 2, 3, 4, we get:

\[
[L^-] = K_1 K_2 [H_2L^+] / [H^+]^2 \quad (6)
\]

But the monoprotic amino acid \(HL\) could act as interacting ligating species and the complexation process could proceeds with proton release as follows:

\[
M + HL \rightleftharpoons ML + H^+ \quad \beta_1^{[HL]} = \frac{[ML][H^+]}{[M][HL]} \quad (7)
\]
\[
\text{M} + 2\text{HL} \rightleftharpoons \text{ML}_2 + 2\text{H}^+ \quad \beta_{1}^{\text{HL}} = \frac{[\text{ML}_2][\text{H}^+]^2}{[\text{M}][\text{HL}]^2} \tag{8}
\]

Where \(\beta_{1}^{\text{HL}}\) and \(\beta_{2}^{\text{HL}}\) are the overall stability constant of the complexes formed from the reaction between HL and M with proton release. In this case, [H] calculated from (2) and substituted in equations (6), (7), we get:

\[
[\text{HL}] = K_1 [\text{H}_2\text{L}]/[\text{H}^+]. \tag{9}
\]

In general, the overall stability constants \(\beta\) can be calculated as:

\[
\frac{n^-}{(1-n^-)[L]} = \beta_i + \beta_z \frac{2-n^-}{1-n^-} [L] + \sum_{i=M}^{j} \left(\frac{i-n^-}{1-n^-}\right) \beta_j [L]^{-1} \tag{10}
\]

Where [L] is the concentration of free ligand and \(n^-\) is the average number of ligand bound per metal ion concentrations \((C_M)^{99-100}\) that expressed as:

\[
n^- = \text{bound ligand}/\text{total metal ligand concentration} \tag{11}
\]

\[
n^- = \frac{L_{\text{bound}}}{C_M} = \frac{L_{\text{total}} - L_{\text{free}}}{C_M} \tag{12}
\]

\[
n^- = \frac{\sum_{i=0}^{n} [\text{ML}]_i}{\sum_{i=0}^{n} [\text{ML}]_i} \tag{13}
\]

The average number of hydrogen ions bound to the ligand \(^{101}\) at different pH Value:

\[
n_A^- = \gamma + \frac{E^0 - N + [\text{OH}^-] - [\text{H}^+] - T_A^0}{T_A} \tag{14}
\]

Where \(E^0\), \(N\) and \(T_A^0\) the concentrations of acid, alkali and ligand added respectively, \([\text{H}^+] = \text{antilog} (-pH)\) and \([\text{OH}] = K_w/[\text{H}^+]\). When \(n_A^-\) versus pH is plotted, the values of pK\(_1\) and pK\(_2\) are equated to the values of pH at which the values of \(n_A^- = 1.5\) and 0.5 respectively.

Now, the total concentration of metal ion \(C_M\), total concentration of \(L_T\) given as:

\[
C_M = [M] + [ML] + [ML_2] + \cdots + [ML_n] = \sum_{i=0}^{n} [ML_i] \tag{15}
\]
Similarly

\[ L_T = [L] + [ML] + 2[ML_2] + \cdots + N[ML_n] = [L] + \sum_{i=1}^{n} i[ML_i] \]  

(16)

The total concentration of \( C_M \) and \( L_T \) are given by:

\[ C_M = [M] \sum_{i=0}^{n} \beta_i [L]^i \]  

(17)

\[ L_T = [L] + M \sum_{i=1}^{n} \beta_i [L]^i \]  

(18)

Where \( ML, ML_2, ML_n \) are the complex species formed in solution by the stepwise addition of ligands to the metal ion and by introducing stepwise stability constants in expression \(^{102-103} n^-\), we get:

\[ n^- = \frac{k_i[ML] + K_{i-1} [ML]^{i-1} + \cdots + nK_n K_2 - K_n [ML]^n}{(M) + K_i[ML] + nK_n K_2 - K_n [ML]^n} \]  

(19)

The concentration of free ligand \( L_{\text{free}} \) is determined directly by the potentiometric titration \(^{104-105} \) of a standard metal perchlorate solution, containing perchloric acid or any acid of a standard sodium nitrate containing nitric acid and ligand of known concentration. After determine the concentration of free ligand \(^{106-107} \) we can calculate \( n^- \) from the equations:

\[ n^- = \frac{L_{\text{total}} - L_{\text{free}}}{C_M} \]  

(20)

The application of equation (9) in the reaction bonding of proton stability constant, we get:

\[ \frac{n^-}{(1-n^-) [L^-]} = \beta_1^{[L^-]} + \beta_2^{[L^-]} \frac{(2-n^-) [L^-]}{(1-n^-)} \]  

(21)

Where

\[ [L^-] = \left( \frac{T_L - T_0 [H^+]}{[H^+]^2 / K_1 K_2 + H^+/K_2} \right) \]  

(22)

A plot of \( \frac{n^-}{(1-n^-) [L^-]} \) against \( (2-n^-) [L^-] \) gives an intercept equal to \( \beta_1^{[L^-]} \) and a slope equal to \( \beta_2^{[L^-]} \), similarly applying eq \(^n\) (9) on the reaction of metal ligand stability constant, we get:

\[ \frac{n^- [H^+]}{(1-n^-) [HL]} = \beta_1^{[HL]} + \beta_2^{[HL]} \frac{(2-n^-) [HL]}{1-n^- [H^+]} \]  

(23)
A plot of \( \frac{n^-(H^+)}{(1-n^-)(HL)} \) versus \( \frac{(2-n^-)(HL)}{1-n^-}(H^+) \) gives an intercept equal to \( \beta_1^{[HL]} \) and a slope equal to \( \beta_2^{[HL]} \). The relationship between the overall stability constant \( \beta_n \) and stepwise stability constant can be given as: \( \beta_n = K_1 K_2 \cdots K_n \). The degree of complexation of the system lies in range \( n^- = 0.5 \) and \( n^- = 1.5 \).

### 1.8.2. Kruck and Sarkar method

P.A. Kruck and Bibudherandra Sarkar\(^{108}\) performed a series of titrations of weak acid, each differing in \( C_A \) (\( C_A \) is the total concentration of ligand) in all forms and used the following expression to calculate the values of \( n_A^- \) at different pH values:

\[
\frac{n_A^-}{\gamma} = \gamma - \left( \frac{\delta C_{NaOH}}{\delta C_A} \right)_H
\]

Sarkar and Kruck recently extended a procedure developed by Osterberg to determine pH in the presence of metal ions by pH. The complexation reactions occurring between \( C_M \) moles of metal ion M, \( C_H \) moles of hydrogen H, and \( C_L \) moles of ligand anion L can be represented by general equilibrium reaction.\(^{109-110}\)

\[
pM + qH + rL \rightleftharpoons Mp Hq Lr
\]

Where (p), (q) and (r) are the stiochiometric quantities of M, H and L respectively. The stabilities of the species formed are represented by the stiochiometric equilibrium constant \( \beta \) expressed in terms of concentrations ionic strength, temperature and pressure:

\[
\beta_{pqr} = \frac{M_p H_q L_r}{m_p h_q l_r}
\]

Where (m), (h) and (l) are the concentration of free metal ion, hydrogen ion and ligand, respectively. The following sets of equations define the total system:

\[
C_M = m + \sum p \beta_{pqr} m^p h^q l^r
\]

\[
C_H = h + \sum q \beta_{pqr} m^p h^q l^r
\]

\[
C_L = l + \sum r \beta_{pqr} m^p h^q l^r
\]

Osterberg showed that the differential quotient of two external coordinates of a titration \( \delta C_H / \delta C_L \) at selected values of pH, and that this function could be used to determine the difference in pH between two points differing in pH:

\[
pL - pL_O = \int_{pH}^{pH_0} \left( \delta C_H / \delta C_A \right)_{H,C_M} dpH
\]
If \( pH_0 \) is selected such that at it \( pL_0 \) is known, e.g., a value low enough that no metal complex formation occurs, then (eq\(^2\)) can be used to find \( pL \) at any other chosen pH. Sarkar and Kruck showed the analogous function \( \delta C_H/\delta C_M \rangle_{H,L} \) could be determined experimentally and used to determine \( pM \) at a selected pH provided that at some reference \( pH_0 \) the value of \( pM_0 \) was known:

\[
pM - pM_0 = \int_{pH}^{pH_0} (\delta C_H/\delta C_M \rangle_{H,L} dpH
\]

Where \( pM = -\log [\text{free metal M}], pL = -\log [\text{free ligand L}], pH = -\log [H^+] \) and \( H_T^+ = \text{moles of } OH^- \) consumed in the titration of the hydrogen ion liberated from the complexation reactions. This is a method which used with computer program to calculate of the stability constant and species distribution of the complexes formed.

1.8.3. Calvin and Wilson method

Calvin and Wilson method\(^1\) is based on measurement of hydrogen ion concentration using pH measurement during titration with alkali of a solution of a chelating agent, in the presence and in the absence of metal ion. The method used to calculate the free ligand concentration (\( pL \)) and the degree of formation of the system (\( n^- \)) and hence the stability constant (\( K_n \)) of the metal ligand complexes present, where the stability constant of the metal ligand complexes \( ML_n \) and \( ML_n = [M] + [ML] + [ML_2] \) and \( ML_n \) was defined by: \( K_n = [ML_n]/[ML_{n-1}][L] \) and that of the ligand–proton complex \( L-H_n \) and is defined by:

\[
K_n^H = [LH_j]/[LH_{j-1}][H]
\]

The overall stability constants are given as for proton ligand stability constant as:

\[
\beta_n = K_1K_2K_4 - K_n
\]

For the determination of \( n^- \) and \( pL \) from Bjerrum equations, the values of K and \( \log K \) was determined by plotting of \( n^- \) against \( pL \) and then the values of \( n^- = 0.5 \) and \( n^- = 1.5 \) given the values of \( K_1 \) and \( K_2 \) respectively.
1.8.4. Irving and Rossotti method

The proton–ligand equilibrium constant for the ligand L under experimental conditions were determined by Calvin Bjerrum pH-titration, as modified by Irving and Rossotti\textsuperscript{112-125} for calculation of \( n_a^- \) and pH from proton-ligand formation. The proton–ligand formation curve was obtained by plotting \( n_a^- \) values against pH. This indicates that the ligand have one dissociable proton. The pK values were estimated from formation curve by noting the pH at which \( n_a^- = 0.5 \) and \( n_a^- = 1.5 \). In Irving and Rossotti method the pH titration of the three sets of mixtures against a carbonate free standard alkali, were performed. These are:

1) Free acid \( \text{A} \)
2) Free acid + ligand \( \text{A+L} \)
3) Free acid + ligand + metal \( \text{A+L+M} \)

On plotting the observed pH against the volume of alkali, we get different trends in the titration curves, the acid curve (A) and the ligand curve (A+L) and a metal complex titration curve (A+L+M) lies below the ligand curve indicating the complex formation. For the present investigation, only Calvin-Bjerrum method, as modified by Rossotti-Rossotti is used, because of their advantages such as comparative simple calculation, less time consuming, economical viable method.

**Calculation of pK and logK**

From the titration curves, the average number of protons associated with the ligand \( n_a^- \) at different pH values is calculated utilizing the acid and ligand curves. The average number of metal ions associated with the ligand \( n \) at different pH values is calculated from the metal ions and ligand titration curves, where the proton–ligand \( n_a^- \) are evaluated as:

\[
 n_a^- = \gamma \cdot \frac{(V_L - V_a)(N + E^0)}{(V_o + V_a)T_L} \quad (33)
\]

Where \( V_a \) and \( V_L \) are the volumes of alkali required to reach the same pH in acid and ligand titration curves. \( T_L \) is the total ligand concentration, \( \gamma \) is the total number of replaceable protons free attached to the ligand molecule, \( N \) is the
normality of the alkali, \( E^0 \) is the initial concentration of free acid and \( V_0 \) is the total volume of the titration solution. The average number of metal ions associated with the ligand \( n^- \) at different pH values is calculated from the metal ions and ligand titration curves using:

\[
n^- = \frac{\text{total number of ligand(L) bound to metal(M)}}{\text{total number of metal present in system}}
\]  \( (34) \)

\[
n^- = \frac{(V_M - V_L)(N + E^0)}{(V_0 + V_L)n^-T_M}
\]  \( (35) \)

And

\[
p_L = \log_{10} \left[ \sum_{n=0}^{nHi} \frac{1}{\beta_{Hi}^{nL}(n^- + pH)} \right] \frac{V_0 + V_M}{V_0}
\]  \( (36) \)

Where \( T_M \) denotes the total concentration of metal present in solution and \( V_M \) the volume of metal ions present in solution and \( \beta_{Hi}^{nL} \) is the overall proton ligand stability constant. There are three most commonly used methods for the calculation of stability constant. Out of these three, we have used two methods i.e. Pointwise calculations and half integral method for calculation of stability constant.

**Method of pointwise calculation**

This method\(^{126-127}\) is used to calculate \( K_1 \) and \( K_2 \) values of the proton ligand formation by using the following expressions:

\[
K_2 = \frac{n^-_A}{(1-n^-_A)[H^+]} \]

\( (37) \)

For monobasic acid:

\[
\log K_2 = pH + \frac{n^-_A}{1-n^-_A} \quad (n^-_A = 0.2 - 0.8)
\]  \( (38) \)

For dibasic acid:

\[
\log K_2 = pH + \log \frac{n^-_A - 1}{2-n^-_A} \quad (n^-_A = 1.2 - 1.8) \]
\[
\log K_2 = pH + \log \frac{n^-_A - 1}{2-n^-_A[H^+]} \]

\( (39a) \)

\( (39b) \)
To calculate $\log K_1$ and $\log K_2$ values of the metal ligand complex using the following expressions:

For monobasic:  \[ \log K_1 = \log \frac{n^- - 1}{1 - n^-} + pL \quad (n^- = 0.2 - 0.8) \]  (40)

The value of $\log K_1$ is determined by using the $n^-$ values in the range of 0.2 to 0.8, and average of $\log K_1$ or $pL$ values were taken, therefore $\log K_1$ equal to $pL$.

For dibasic:  \[ \log K_2 = \log \frac{n^- - 1}{2 - n^-} + pL \quad (n^- = 1.2 - 1.8) \]  (41)

The value of $n^-$ is selected in the range of 1.2 to 1.8. The average values of $\log K_2$ or $pL$ which taken where $\log K_2$ equal to $pL$. The accuracy of $\log K$ was $\pm 0.002$ unit.

**Half integral method of calculation**

In the case of proton-ligand, we can calculate the stability constant by plotting $n^-_A$ against pH, the value of pH where $n^-_A=1.5$ and $n^-_A=0.5$ corresponds to the values of $pK_1$ and $pK_2$ respectively, and in the case of a metal ligand curve by plotting $n^-$ against $pL$, calculate $\log K_1$ and $\log K_2$ from the formation curve by the known values of $pL$ at which $n^- = 0.5$ and $n^- = 1.5$ corresponds to the values of $\log K_1$ and $\log K_2$ respectively.

**Method of least squares**

Irving and Rossotti showed the better set of stability constants from different experimental data that can be solved by least squares method after an algebraic transformation. Moreover when the difference between $\log K_1$ and $\log K_2$ was less than 1.8, the exact values were evaluated by this method. For a system consisting of (1:1) and (1:2) complex species, the following expression was employed:

\[ \frac{n^-}{(n^- - 1)} = \frac{(2-n^-)[L]}{(n^- - 1)} \cdot K_1K_2 - K_1 \]  (42)
A plot of \( \frac{n^-}{(n^- - 1)(L)} \) against \( \frac{(2-n^-)[L]}{(n^- - 1)} \) give the best straight line with slope= \( K_1K_2 \) and the intercept \( K_1 \). The method was also utilized to confirm the presence and absence of 1:2 complex species \(^{130}\). Nayan and Dey\(^ {131}\) have also attempted to simplify the approach for calculating various parameters required for stability constant.

1.9. Ternary complexes (mixed-ligand complex systems)

Ternary complexes\(^ {132-133}\) formed between metal ion and two different types of biological ligand, namely heteroaromatic nitrogen bases and amino acids may be considered as models for substrates-metal ion-enzyme interactions and other metal ion biochemical interactions. Among these compounds, metal-ligand complexes are known to play a significant role either in naturally occurring biological systems or as pharmacological agents such as antitumor, anticandida, antimycobacterial, antimicrobial activity\(^ {134}\). So, that much attention has been paid recently to the study of ternary complexes of transition metals with molecules of biological and pharmaceutical interest. Furthermore, it has been suggested that the presence of metal ions in biological fluids, have a significant effect on the therapeutic action of drugs. Potentiometric titration is more suitable for the study of ternary complexes which may be evaluated by the following equilibrium:

\[
pM + qH + rA + sB \rightleftharpoons Mp Hq Ar Bs
\]

Where M is the metal ion, H is the proton, A and B are the ligands. The stability constant of the ternary complexes may be represented as following:

\[
\log \beta_{pqr} = \frac{[M_p H_q A_r B_s]}{[M]^q[H]^r[A]^s[B]^t}
\]

The relationship between the stability constants for ternary complexes to their binary ones\(^ {135-139}\), represented by the equilibria:

\[
M + B \rightleftharpoons MB \quad K_{MB}^M = \frac{[MB]}{[M][B]}
\]

\[
MA+ B \rightleftharpoons MAB \quad K_{MAB}^{MA} = \frac{[MAB]}{[MA][B]}
\]
The different between the stability of the ternary and binary complexes, show the tendency of the formation of ternary species and given as:

\[ \Delta \log K = \log K^M_{MA} - \log K^M_{MB} \]  
(47a)

\[ = \log K^M_{MAB} - \log K^M_{MA} \]  
(47b)

OR

\[ \Delta \log K = \log \beta_{i11} - (\log \beta_{01} + \log \beta_{i0}) \]  
(48)

A statistical evaluation of ternary complexes formation may be calculated using:

\[ \log_{2} \beta_{i11} = \log_{2} \beta_{01} + \log_{2} \beta_{i0} \]  
(49)

\[ \beta_{01} = 2^{1/2} \beta_{01} \]  
(50)

The difference between the constant from experimental data and those calculated statistically indicates the possibility of ligand–ligand interaction.140.

1.9.1. Mechanism of complexation

The stability constant of ternary chelates may be discussed according to two methods of equilibrium, in the first method, the ternary complexes formation was considered according to the following equilibria:

\[ M + L_P + L_S \rightleftharpoons ML_P L_S \]

\[ \log \beta_{MML_P L_S} = \log [ML_P L_S] - \{\log [M] + \log [L_P] + \log [L_S]\} \]  
(51)

Since

\[ \log K^M_{ML_P} = \log [ML_P] - \{\log [M] + \log [L_P]\} \]  
(52)

\[ \log \beta^M_{ML_P L_S} = \log [ML_P L_S] - \{\log [ML_P] + \log K^M_{ML_P} + \log [L_S]\} \]  
(53)

In the second method, the \( ML_P L_S \) chelate was considered to be formed in stepwise manner:

\[ ML_P + L_S \rightleftharpoons ML_P L_S \]

\[ \log \beta^M_{ML_P L_S} = \log [ML_P L_S] - \{\log [ML_P] + \log [L_S]\} \]  
(54)

Similarly

\[ ML_S + L_P \rightleftharpoons ML_S L_P \]

\[ \log \beta^M_{ML_S L_P} = \log [ML_S L_P] - \{\log [ML_S] + \log [L_P]\} \]  
(55)

The overall stability constant \( \beta^M_{ML_P L_S} \) is connected with \( K^M_{ML_S L_P} \) and \( K^M_{ML_P L_S} \) by the
following equations:

\[ \log^{M}_{PMLP_{PLS}} = \log^{MLP}_{MLP_{PLS}} + \log^{M}_{MLP} \]  
(56)

\[ \log^{M}_{PMLSLP_{P}} = \log^{MLS}_{MLSLP_{P}} + \log^{M}_{MLS} \]  
(57)

The relative stability of ternary complex \( ML_{P}L_{S} \) as compared with that corresponding binary complex \( ML_{S} \) can be quantitatively expressed in different ways in terms of \( \Delta \log K \), according to the secondary ligand:

\[ \Delta \log K = \log^{MLP}_{MLP_{PLS}} - \log^{MLS}_{MLS} \]  
(58)

On statistical considerations\(^{141} \), \( \Delta \log K \) is expected to be negative, when the ligand combines with a free metal ion, it has more coordination positions available for binding than when it combines with a metal ion already bound to another ligand. Thus, the value of \( \Delta \log K \) depends on the coordination number of the metal ion and the denticity of the ligand. It is also affected by non-statistical factors like the nature of the ligands \( L_{P} \) and \( L_{S} \). A less negative value of \( \Delta \log K \) indicates increased stability of the ternary complex as a result of other factors that affect the statistical operation. Other way, there are two reactions represent the following overall equilibrium:

\[ ML_{P} + ML_{S} \rightleftharpoons ML_{P}L_{S} + M \]

And hence

\[ \Delta \log K_{M} = \log^{MLP}_{MLP_{PLS}} - (\log^{MLP}_{ML_{P2S}} + \log^{MLS}_{ML_{S2P}}) \]  
(59)

\( \Delta \log K \) is negative values which means that ternary complexes of these ligands are less stable of corresponding binary one, and if positive values of \( \Delta \log K \) which means the ternary complexes of these ligands are more stable of corresponding binary ones\(^{142} \). The quantitative stabilization of ternary complexes can also be in terms of disproportionation constant \( X \) as defined by the equation:

\[ ML_{P2S} + ML_{S2P} \rightleftharpoons 2ML_{P}L_{S} \]

\[ X = [ML_{P}L_{S}]^{2} / [ML_{P2S}][ML_{S2P}] \]  
(60)

\[ \log X = 2\log^{M}_{PMLP_{PLS}} - (\log^{M}_{ML_{P2S}} + \log^{M}_{ML_{S2P}}) \]  
(61)

OR \( \log K_{\text{repr.}} = \log^{MLS}_{MLP_{PLS}} - \log^{MLP}_{ML_{P2S}} + \log^{MLP}_{ML_{P2S}} - \log^{MLS}_{ML_{S2P}} \)  
(62)

The more positive value than those expected indicate the marked stability of ternary complexes. The \( \log X \) values can only indicate the coordination tendency
of secondary ligand\textsuperscript{143} towards $ML_S$. On statistical considerations, the value of $\log K_{\text{reprop}} = 0.6$ doesn’t indicate that the absolute stability of the complex, but its relative stability with respect to the complexes $ML_{2P}$ and $ML_{2S}$. In the case of electrostatic or stereochemical reasons the stability of $ML_{2P}$ or $ML_{2S}$ can be either less or more of $ML_P L_S$, the value of $\log K_{\text{reprop}}$ is higher than 0.6. Other parameter is percent Relative Stabilization\textsuperscript{144} ($%\ R.S.$) to quantify the stability of a ternary complex and that may be defined as:

\[
(\% R.S) = [(\log K_{ML_P L_S}^{ML_P} - \log K_{ML_P}^{ML_P} / \log K_{ML_P}^{ML_P})] \times 100
\]  

\textbf{1.9.2. Calculation of stability constant of ternary complexes}

There are some methods used to calculate of the stability constant of ternary complexes:

\textit{Stepwise method}

The stepwise equilibria in solution would be confirmed when the mixed ligand curve could be superimposed over the binary $ML_P$ or $ML_S$ titration curve. The method of Thomson and Loraas\textsuperscript{94} for calculation of stepwise stability constants is widely used. The stabilities of the mixed–ligand complexes can be calculated by the replacement of $M=L_P=L_S$ by $ML_P$ or $ML_S$ in the following expression.

i) For monobasic acid as primary ligand

\[
K_{ML_P L_S}^{ML_P} = \frac{T_{M=AX}}{A^2 X}
\]  

Where

\[
A = \frac{T_{LS}-T_{OH}}{[H^+]/K_1}
\]

And

\[
X = \frac{[H^+]}{K_1} + 1
\]

ii) For dibasic acids as secondary ligand

\[
K_{ML_P L_S}^{ML_P} = \frac{T_{M=AX}}{A^2 X}
\]  

Where

\[
A = \frac{2T_{R=OH} - 2[H^+] + [OH^-]}{[H^+]^2 / K_1 + [H^+]^2 / K_2}
\]
And
\[ X = 1 + \frac{[H^+]}{K_1} + \frac{[H^+]^2}{K_1 K_2} \]

**Simultaneous equilibria**

When the mixed ligand curve does not coincide with either of the simple binary curves \((ML_P\) or \(ML_S\)), the equilibria involved is simultaneous one. Irving and Rossotti\(^{145}\) and Santappa and Ramamoorthy\(^{115}\) have developed a number of equations for the evaluation of stability constants of mixed complexes involving number of ligands. Following expressions (modification of Thomson and Loraas) are utilized for the determination of stability constants of ternary complexes.

i) For monobasic-monorbasic acids \((T_M=T_{L_S}=T_L)\)

\[
K_{ML_{S}L_P} = \frac{[T_M-(0.5[A]X)]}{0.5^9[A]^9 X} \tag{66}
\]

Where
\[
A = \frac{2T_M+[T_{OH}-[H^+]]}{2[H^+] K_1 K_2} \quad (T_M=T_R=T_L)
\]

\[
X = 1 + \frac{2[H^+]}{K_1+K_2}
\]

ii) For dibasic -dibasic acids

\[
K_{ML_{S}L_P}^ {ML} = \frac{[T_M-[0.5[A]X]}{0.5^9[A]^9 X} \tag{67}
\]

Where
\[
A = \frac{4T_M+[T_{OH}-[H^+]]}{2[H^+] K_2 K_2 + \frac{4[H^+]^2}{(K_1 K_2)+(K_1 K_2)^2}} \quad (T_M=T_{L_P}=T_{L_P})
\]

\[
X = 1 + \frac{2[H^+]}{K_2+K_2} + \frac{2[H^+]^2}{(K_1 K_2)+(K_1 K_2)^2}
\]

iii) For monobasic-dibasic ligands \((T_{L_P}=T_{L_S}=T_{L_P})\)

\[
K_{ML_{S}L_P}^ {ML} = \frac{[T_M-0.5[A]X]}{0.5^9[A]^9 X} \tag{68}
\]

Where
\[
A = \frac{3T_M+[T_{OH}-[H^+]]}{2[H^+] K_2 K_2 + \frac{2[H^+]^2}{K_1 K_1 K_2}}
\]

\[
X = 1 + \frac{2[H^+]}{K_2+K_1} + \frac{[H^+]^2}{K_1 K_2}
\]

Where \(T_M=\) Concentration of total metal ion.
Introduction

\[ T_{Lp} = \text{Concentration of primary ligand.} \]
\[ T_{Ls} = \text{Concentration of secondary ligand.} \]
\[ P = \text{Initial concentration of HClO}_4. \]

And \[ T_{OH} = \text{Concentration of alkali [NaOH].} \]

\[ K_1, K_2 \text{ and } K_1^- \text{ and } K_2^- \] are the first and second dissociation constants of two ligands. In the present investigation, it was observed that in all mixed ligand systems, the mixed curve did not coincide with either \( ML_p \) or \( ML_s \) curve since from the beginning existence of complex species was confirmed by simultaneous equilibria.

**Calculation of stability constants of the ternary complexes using computer programme (SCOGS)**

The SCOGS program \(^{146-150}\) was used to calculate the stability constant of ternary complexes; the data obtain using potentiometrically titration. The program employs nonlinear least square method to calculate simultaneous or individual association constant for any of the species formed in system containing up to two metals and two ligands. It used to calculate metal ion hydrolysis constant, stability constant of polynuclear species, acid dissociation constant, stability constant for mixed complexes containing two different ligands or two different metal ions, in the mixture of two metals \( M_1 \) and \( M_2 \) and two ligands \( L_p, L_s \) association constant can be calculated with the programme for any species \( j \) which can be designed by the general formula: \[ M_{1j}M_{2j}L_{p}L_{sj}(OH)_{Nj} \] (69)

Where \( M_{1j}, M_{2j}, L_p, L_s \) are positive integers or zero and \( N_j \) is a positive integer for hydrolysed species, zero or negative integer for protonated species. In the practical overall formation constant \( \beta_j \) given by the expression:

\[
\beta_j = \frac{M_{1j}M_{2j}L_{p}L_{sj}^{(OH)}_{Nj}}{[M_{1j}^1] [M_{2j}^2] [L_p^1] [L_s^1] [OH]^{Nj}},
\]

(70)

Where square brackets \([ \ ]\) denotes concentration and small bracket \(( \ )\) denotes activities.
1.10. Aim of the present work

An exhaustive literature survey on binary and ternary complexes of biological active ligands shows that no attempt has been made so far on the determination of stability constant. The present work was undertaken to determine systematically the stability constants of binary as well as ternary system of some transition metal ions with drugs and amino acids (glycine). The aim is to study formation stability of ligands and stability constants of binary and ternary complexes of cobalt, nickel, copper and zinc (transition metal ions) with medicinally important drugs and amino acid in aqueous media at room temperature in ionic strength 0.01 M (NaClO₄), with different ratio of ligands for binary complexes (1:1), (1:2) and ternary complexes (1:1:1), (1:1:2) and (1:2:1). Metal ions so chosen on the basis of their physiological and pharmacological importance. The drugs and amino acid used in the present study are as follows:

1) Ciprofloxacin.HCl
2) Pantoprazole Sodium
3) Gabapentin
4) Chloramphenicol
5) Ceftriaxone Sodium
6) Atenolol

And amino acid such as glycine

Literature survey shows that the compounds contains donor group are extensively used in biology and medicinal chemistry. The metal chelates of donor groups are also used nowadays. In view of the great analytical, biological, industrial and manifold uses of donor groups complexes with metals. The stability of these chelates is therefore, an important factor in determining the effectiveness of these chelates in above mentioned fields. The main objective of the present investigation was to study coordination behaviors of drugs, amino acids and their binary and ternary metal complexes in the solution. Effect of substituent’s on formation constants of ligand and their chelates, effect of ligand basicity on
the stability constant of complexes to evaluate the correlation between stability constant of complexes and the atomic number of metals, and to calculate the equilibrium constants by knowing the distribution of various species at different pH values.
References


35. Peter Gans. Antonio Sabatini · Alberto Vacca, simultaneous calculation of equilibrium constants and standard formation enthalpies from calorimetric


Introduction


75. J. Bjerrum. Metal amine formation in aqueous solutions. P.Haase and Son, Copenhagen. 1941.


89. James R Hanson, Chemistry and Medicines-An Introductory Text, the Royal Society of Chemistry, 2006.


113. Ahmed A. A. Boraei, Fouad Taha, and Ali H. Mohamed, Medium effect and Thermodynamic studies for the proton-ligand and metal-ligand formation


134. Fatma S. M. Hassan, Formation of mixed ligand complexes of Ni (II), pd (II), and pt(II) involving salicylidine-3-amino-1,2,4-triazole (schiff base) as a primary ligand and cysteine as secondary ligand, *Arab J. Sci. Eng.* 30, 1A, 2005.


