CHAPTER 1
INTRODUCTION
The science of coordination chemistry is passing through such a state of rapid advance that many of the ideas and theories of past two decades have already been either discarded or modified. Coordination compounds play an important role in numerous chemical and biological systems. Their importance becomes clear when one realises that chlorophyll, vital to photosynthesis in plants, is a magnesium complex and haemoglobin which carries oxygen to animal cells is an iron complex. Studies on enzymes have shown that the site of reaction in biological systems is frequently a complexed metal ion. Several industrial processes depend directly on catalysis by metal complexes. The 1963 Nobel Prize in chemistry awarded jointly to Dr. K. Zeigler of Germany and Professor G. Natta of Italy, was for development of aluminium and titanium complex as a catalyst for the low pressure polymerisation of ethylene. Coordination compounds have also played a very important role in biological activities for removal of undesirable and harmful metals from living organisms. Number of coordination compounds are used for seperation of lanthanides, water softening, ionexchange resins, electroplatening, dyeing, antioxidants etc.

1.1 Proteins, Peptides And Amino Acids.

"There is present in plants and in animals, a substance which is without doubt the most important of all the known substances in living matter, and without it life would be impossible on our planet. This material has been named protein."

So wrote Gerard Johannes Mulder, a Dutch Agricultural Chemist. Proteins, the keystones of life, are the most complex substances known to man and their chemistry is one of the great challenges in modern science for more than a century; thousands of different proteins go into the make up of a living cell. They perform thousands of different acts, in the exact sequence, which cause the cell to live. Proteins are giant molecules of great size, complexity and diversity. Each appears to be designed with high specificity for its particular task. Clear picture of each aspect of proteins is yet to be known. High polymer chemistry is now coming forward with answers to some of the pressing questions of biochemistry. The most important of proteins are enzymes. The
Hormones are also proteins. Some proteins are antibodies in the blood, which defend the organism against viruses. Finally, genes, the basic unit of heredity, are believed to possess particular type of proteins called as nucleoproteins. To learn, which proteins are present in living systems, to examine their chemical structures and to explain their biological functions in terms of their structures, these are among the most fundamental problems of modern biochemistry.

Sixty years ago, it was discovered by the German Chemist Emil Fischer, that proteins consist of long chains of amino acids residues. Long chain of amino acids residues are called polypeptide chains. The chains are usually very large; for example, in the molecule of ovalbumin, the main protein constituent of white of egg, about 400 amino acid residues form a single polypeptide chain.

Amino acids are formed by hydrolysis of proteins and have certain structural features in common. Each has an acidic carbonyl group (-COOH) and a basic amino group (-NH₂) or imino group (>NH). Both the acidic and basic groups are attached to the same carbon atom, the so-called α-carbon atom. Since a carbon atom has four chemical valencies, this same α-carbon has two other units linked to it; one of these is invariably a hydrogen atom. What distinguishes the amino acids from one another is the fourth group attached to α-carbon. This group, the so-called side chain, differs in each amino acid. In a protein molecule they are linked by combination of the carboxyl group of one unit with the amino group of the next. In the process of combination two hydrogen atoms and an oxygen atom drop out in the form of a water molecule and link becomes -CONH. This linkage is called peptide bond. A group of linked amino acids is known as a peptide, 'two units form a dipeptide, three units a tripeptide and so on.'

The simplest amino acid, glycine, was isolated in 1820 by French Chemist Henry Braconnot. The list of known amino acids from proteins has now grown to 22. In every protein, amino acid, except glycine can exist in two geometrical forms, one being the mirror image of the other, by convention these are designated as the 'L' and 'D' forms. Only the 'L' type of amino acids are obtained by the hydrolysis of proteins.
DNA (makes partial copies of itself) → Messenger RNA (moves out of the nucleus into the cell cytoplasm) → Protein synthesis → Mixture of amino acids

acids, alkalis or enzymes (Hydrolysis) → Mixture of amino acids

1.1.1 Peptides

In the body, peptides are formed during the initial digestion of the proteins and inside the cells by synthesis from amino acids.

Carnosine and anserine are dipeptides found in the voluntary muscles. An important tripeptide is glutathione which contains glutamic acid, cysteine and glycine.

(γ glutamyl cysteinyl glycine)

\[
\begin{align*}
\text{CH}_2 & - \text{C} - \text{N} - \text{C} - \text{N} - \text{CH}_2 - \text{COOH} \\
\text{CH}_2 & - \text{NH}_2 \\
\text{COOH} & \\
\text{glutamic acid} & \\
\text{SH} & \\
\text{cysteine} &
\end{align*}
\]

Other peptides of biological importance containing many amino acids are glucagon, oxytocin, vasopressin, MSH, angiotensin and adreno-corticotropic hormone (ACTH).^3

Biologically Important Peptides^4

Some small peptides which have significant biological activity are formed as a result of hydrolysis of large proteins while some are formed by synthesis.

1. *Glutathione*: This is a tripeptide consisting of glutamic acid, cysteine and glycine. By virtue of easy dehydrogenation it gets converted to disulphide form and function in oxidation-reduction systems.

2. *Camosine*: This is a dipeptide of β-alanine & histidine and anserine made up of β-alanine and methylhistidine which are water soluble dipeptides of voluntary muscle.

3. *Bradykinin*: Bredikinin (9 amino acids) or Kallidin I and Kallidin II (10 amino acids) have relaxant effects on smooth muscle.

4. *Oxytocin and Vasopressin*: Found in pituitary gland, these are cyclic peptide hormones made up of 8 amino acids. Oxytocin acts on uterine muscle before parturition and in the ejection of milk while vasopressin influences reabsorption of water from the distal and collecting tubule of the kidney.
5. **Angiotensins**: The enzyme renin is released from kidneys and acts on globulin fraction of plasma to release a peptide Angiotensin I (10 aminoacids) which has only a slight effect on blood pressure, Angiotensin I is then converted to Angiotensin II by splitting off 2 amino acids (which has 8 amino acid) which has greater effect on blood pressure. Besides its pressor effect, Angiotensin II stimulates thirst, dilation of blood vessels of voluntary muscle & brain and increases aldosterone secretion. Removal of one residue aspartic acid from Angiotensin II results in formation of Angiotensin III (7 amino acids) which plays role in pathology of hypertension.

6. **Gastrin, Secretin and Pancreozymin** are gastrointestinal peptides which act as hormones which stimulate secretion of bile and other enzymes of digestive juices.

7. β-Corticotropin (ACTH), βMSH are peptides which are hormones.

8. **Antibiotics**: Penicillin, gramicidin, polymyxins, bacitracins, actinomycin and chloramphenicol are all peptides which act as antibiotics.

9. **Brain Peptides**: Certain brain cells have receptors that bind opiates like morphine and have been termed endorphins (endogeneous morphine). *Dynorphin* is a peptide of 13 aminoacids which is called *superopioate* since it is significantly potent.

Peptide fragments from brain that reduce intestinal motility are met-enkephalins and leu-enkephalin both being pentapeptides.

**Bradykinin**: This is a nonapeptide which is one of the most potent pain producing substances; has been shown to mediate production of prostaglandin $E_2$ from arterial walls. The decapeptide substance $P_3$ is found in dorsal horns of spinal cord and the intestine. It is concerned with voluntary movement and pain relief. There are several hormones such as somatostatin (growth hormone release inhibiting factor) and thyroid releasing hormone (TRH), are all originated in brain.

This wide range of peptides are related with several interlinked brain systems. They are potent pain killers or are able to potentiate the action of pharmacologic agents influencing patterns of behaviour.
Biomedical Importance

Peptides are of immense biomedical interest, particularly in endocrinology. Many major hormones are peptides and may be given to patients to correct corresponding deficiency states (e.g. administration of insulin to patients with diabetes mellitus). Some peptides act in the nervous system, either as neurotransmitters or as neuromodulators. Certain antibiotics are peptides (e.g. valinomycin and gramicidin A), as are a few antitumor agents (e.g. bleomycin). The dipeptide aspartame serves as a sweetening agent in many beverages. Rapid chemical synthesis and recombinant DNA technology have facilitated the manufacture of substantial amounts of peptide hormones, many of which are present in the body in relatively minute concentrations and thus difficult to isolate in quantities sufficient for therapy. The same technology allows the synthesis of other peptides, also available from natural sources in only small amounts (e.g. certain viral peptides and proteins), for use in vaccines.

Some Peptides Have Intense Biological Activity

In addition to the peptides formed as products of the partial hydrolysis of protein molecules, many peptides occur in free form in living matter, not associated with protein structure. Most interesting is the fact that many such free peptides have intense biological activity. For example, a number of hormones are known to be peptides or polypeptides. The hormone insulin, secreted by the B cells of the pancreas, is a chemical messenger carried by the blood to other organs, especially the liver and muscles, where it becomes bound to receptors on cell surfaces and stimulates the capacity of these cells to use glucose as a metabolic fuel. Insulin contains two polypeptide chains, one having 30 aminoacid residues and the other 21. Other polypeptide hormones include glucagon, a pancreatic hormone that opposes the action of insulin, and corticotropin, a hormone of the anterior pituitary gland that stimulates the adrenal cortex. Corticotropin has 39 aminoacid residues.
Some hormones have much shorter peptide chains. Among them are oxytocin (nine aminoacid residues), a hormone secreted by the posterior pituitary that stimulates uterine contractions; bradykinin (nine residues), a hormone that inhibits inflammation of tissues; and thyrotropin-releasing factor (three residues), which is formed in the hypothalamus and stimulates the release of another hormone, thyrotropin from the anterior pituitary gland. Especially noteworthy among short peptides are the enkephalins, formed in the central nervous system. When enkephalins bind to specific receptors in certain cells of the brain, they induce analgesia, deadening of pain sensations. Some extremely toxic mushroom poisons, such as amanitin, are also peptides, which may be regarded as “chemical warfare” agent, made by some species of microorganism but toxic to others.

It is most remarkable that these peptides have such potent biological effects, despite the fact that the aminoacids of which they are composed are harmless, nontoxic substasnce. Clearly, it is the sequence of aminoacids in peptides and polypeptides that gives them their striking biological effects and specificity.

1.2 Metal Chelates

The stability of transition metal-aminoacid complexes in solution has been extensively investigated and a number of crystal structures have been determined. The optimised geometries of 1:1 complexes of α-alanine with Cu²⁺ and Ni²⁺ have been confirmed.

La(III) and Ce(III) are known to form complexes with aminoacids. The formation constants of La(III), Pm(III) and Nd (III) complexes with glycine are known. Stabilities and thermodynamics of Uranyl(II) & Thorium(IV) complexes of DL-nor-leucine have been studied. The complex formation of Uranyl (II) and Vanadyl(II) with DL-tryptophane have been studied employing potentiometric technique and overall changes in thermodynamic functions have been calculated. Irving–Rossotti titration technique has been used by Patel et al to determine the stability constants of aminoacid complexes of trivalent lanthanones. The formation constants of trivalent lanthanide-amino acid complexes follow the order:

La < Ce < Pr < Nd < Sm < Gd.
1.3 Metal-Ligand Complexes In Solution
And Their Stability Constants

Stability constants of complexes have played an important tool for determining the thermodynamic parameters such as enthalpy, entropy, free energy etc.

Rossotti and Rossitti\textsuperscript{14} have defined a complex as a specie formed by the association of two or more species each capable of independent existence; when one of the species is a metal ion, the resulting entity is known as metal complex.

The term ligand is sometimes applied to the particular atom in the molecule by means of which molecule is attached to the central metal atom or it may be applied to the molecule as a whole. Some ligands are attached to the metal atom by more than one donor atom in such a manner as to form heterocyclic ring. This is known as chelation. The chelates have been extensively studied in solution as well as in solid state by many workers because of their remarkable properties and high stability. The extensive work in coordination complexes has been made possible with the help of various experimental techniques and has led to a number of empirical conclusions which have been detailed by Martell and Calvin\textsuperscript{15}.

Considerable research work has been done on the study of complexes in solution in the last four decades. The development in the field was initiated by Bjerrum's Dissertation\textsuperscript{16} published in 1941. Calvin, Irving, Rossotti, Martell and Schwarzenbach have made important contributions to the rapid progress in our understanding of metal complexes in aqueous as well as in mixed solvents. A much stronger emphasis is laid on mixed ligand, protonated, polynuclear and outershare type complexes (Beck and Nagypal, 1990); these species are frequently regarded as somewhat exotic, but their existence must be taken into consideration in general\textsuperscript{17}.

The stability of complexes in solution is governed by the nature of central atom and the ligands. The most important characteristics of central atom which influence the stability of complex compound are the degree of oxidation, the radius and electronic structure. In the case of complexes with monoatomic ligands, stability is dependent on the same characteristics in the ligand as considered for the cation. The strength of binding for ligand molecules and polyatomic ions depends, in addition, on the nature of atoms.
directly linked to the central atom and on the particular features of the structures of the ligand molecule or ion.

A complex formation is favoured by negative enthalpy and positive entropy changes. It is very difficult to predict the contribution of these terms because the solvation of the constituents of the complex also must be taken into consideration. According to Williams\textsuperscript{18} the entropy term is usually favourable when the ligand is anionic and is generally unfavourable in case of neutral ligands. Enthalpy changes in complex formation are generally determined by:

(i) Temperature Coefficient Method and (ii) Microcolorimetry.

The stability of complexes also depends upon the size and number of chelating rings. The size of the chelating ring and number of rings formed on chelation are determined by the structure of chelating agent. Hence the stability of chelate depends upon both the factors. Ley\textsuperscript{19} has concluded from his work on aminoacid chelates that five and six membered rings are most stable. Since then much evidence has accumulated to prove that all chelates have either five or six membered rings. Pfeiffer\textsuperscript{20} observed that, in general, the five membered ring is more stable when the ring is entirely saturated, but when one or more double bonds are present, the six membered ring is favoured. Schwarzenbach and Ackerman\textsuperscript{21} have observed that there is decrease in chelate stability with the increase in ring size. Steric influence of the ligand-$\beta$-diketone and its tetramethyl analog are studied by Yutaka et al\textsuperscript{22}. Saha and Sinha\textsuperscript{23} have reported increase in the value of formation constant with the change of alkylgroup from methyl to n-butyl in R$_2$Sn (IV) possibly due to increase in the electrophilic character of tin with the increase in the length of alkyl chain.

Chelation as well as stability is governed by nature of metal. Nature of metal ion also plays an important role in chelation and stability of complexes. Stability order of metal complexes of transition metal ions is found by Irving and Williams\textsuperscript{24} by comparing the ionic radius and second ionisation potentials of the metal ions because it is valid for most nitrogen and oxygen donor ligands.

This order was rationalised as:

$\text{Mn}^{2+} < \text{Fe}^{3+} < \text{Ni}^{2+} < \text{Cu}^{2+} > \text{Zn}^{2+}$

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1.4 Correlation Between The Basicity Of The Ligand And The Stability Of Complexes.

In most cases, the complex formation is a complexation between metal ions and protons. It is therefore, reasonable to expect that there is some correlation between the stability constant of the complex and the acidic dissociation constant of the conjugated acid of the ligand. Larson\textsuperscript{25} found a linear relationship between the corresponding constant for the complexes of Silver (I) with organic amines. Subsequently similar correlations are found in many complex systems. The ligand may affect the chelating tendency in two possible ways:

i) it may influence the basicity of the donor groups by inductive and resonance effects or / and

ii) the addition of groups on the ligand may be purely statistical.

Steric effects prevent the ligand ions or molecule from acquiring the orientations about the central metal ion most favourable for chelation. In certain cases a linear correlation is found between the Hammett’s constants for the functional groups in ligand and the logarithms of the stability constants of the complexes. May and Jones\textsuperscript{26} have applied Hammett’s equation to the complexes of substituted benzoic acids. Irving and Da Silva\textsuperscript{27} introduced a stability factor ‘sf’ which is a measure of the stabilisation due to bonding.

In the present work some peptides are used as ligands and their chelating nature in aqueous and nonaqueous solvent is studied. It would be appropriate to understand the knowledge about the structure of water and its modification by organic solvent.

1.5 Structure Of Water

Liquid water is regarded as a mixture of quasicrystalline modification\textsuperscript{28} in which each molecule is capable of taking part in four tetrahedrally-oriented hydrogen bonds, and a nonhydrogen bonded molecular species, the properties of which are not clearly defined. According to the gas hydrate type model put forward by Frank and Quist\textsuperscript{29}, the quasi-crystalline structure resembles a clathrate lattice which is stabilized by the presence of interstitial monomeric H\textsubscript{2}O molecules. The model of Nemethy and Scheraga\textsuperscript{30}, on the other hand, provides for a distribution of ice-like clusters dispersed in a denser, non–hydrogen bonded form.
of water. Both models have been partially successful in accounting for the observed enthalpies and entropies of evaporation of hydrocarbons from aqueous solution. The introduction of a nonpolar solute molecules into liquid water is believed to be associated with a shift in the structural equilibrium in the direction of greater structuredness or ice likeness of the water. This effect of nonpolar solutes on liquid water is sometimes referred to as 'hydrophobic hydration' to distinguish it from the normal hydration which arises from strong interaction between molecular or ionic solutes and water.

Frank and Wein\textsuperscript{31} have argued that the formation of hydrogen bonded dimer of two $\text{H}_2\text{O}$ molecules makes it to form additional hydrogen bonds with other $\text{H}_2\text{O}$ molecules because of contribution (to partially covalent hydrogen bond) of a resonance form having a partial charge separation. In other words, the formation of hydrogen bonds in the liquid is a cooperative phenomenon i.e. the bonds are not made and broken singly but several at a time, thus producing short lived 'clusters' of highly hydrogen bonded regions surrounded by nonhydrogen bonded molecules. These clusters may be expected to be compact and nearly spherical in shape. On the basis of Frank – Wein assumptions, no appreciable amount of small aggregates (dimers, trimers etc.) will exist, only clusters and monomers would exist.

1.6 AIM OF THE PRESENT WORK

Metal –ligand stability constants of the complexes of transition metal ions, lanthanides and nuclear metal ions are studied by many workers\textsuperscript{15,17}. The efficiency of chalcone as an analytical reagent for spectrophotometric estimation of a number of metal cations has been recognized by Shyamsundar\textsuperscript{32}. Narwade et al\textsuperscript{33} have studied the stability constant of transition metal ion with some substituted chalcones. Sawalakhe and Narwade\textsuperscript{34} have investigated the metal-ligand stability constants of Fe(III), Cr(III) and Al(III) metal ions with some substituted pyrazoles. Mandakmare and Narwade\textsuperscript{35} have studied the metal-ligand stability constants with some substituted coumarins by potentiometric and spectrophotometric technique. Tandon et al\textsuperscript{36}, have studied Pt(IV) complexes with some substituted aryl-thioureas spectrophotometrically. Adiabatic
compressibility and hydration number of some substituted aminoacids in different percentage of non-aqueous and aqueous media are studied by Pankanti and Jahagirdar. Swami and Lingaith have reported the formation constants of some bivalent metal chelates of 2-hydroxy-5-methyl chalcone at 0.10M ionic strength in 70% ethanol-water mixture. Mahajan and Narwade have studied the influence of dielectric constants of medium on Cu(II)-aminosulphonicacid complexes at 0.10M ionic strength. Khobragade and Narwade have studied the interaction between Fe(III) and 1,2, dihydroxy benzene-3, 5-disulphonicacid in aqueous medium potentiometrically and spectrophotometrically Narwade et al. have reported (i) formation constants of lanthanide complexes with some substituted sulphanicacids. (ii) the interactions of thorium ion with some substituted pyrazolines using Calvin–Bjerrum technique. (iii) the stability constants of Cu (II) chelates with some substituted 1,3-propanediones at 0.10M ionic strength and (iv) the conditional stability constants of Cu(II) complexes with some substituted chalones and isoxazolines. Zaheer and Dhule have investigated the transition metal ion complexes with 4-amino 2, 6-dihydroxy pyrimidine. Most of the above research work is carried out in our research laboratory.

However a detailed study of complexes with peptides under identical set of experimental conditions which would cover manifold aspect of complexation is still lacking.

The present work is, therefore, undertaken to make a systematic study of the chelates. A systematic study of stability constants of binary complexes of some peptides with Mg(II); Co(II), Ni(II), Cu(II); Fe(III), Cr(III), Al(III); Ce(III), Nd(III); UO₂(II) & Th(IV) metal ions have been performed by pH-Metric Method. Some of the systems have also been studied spectrophotometrically for comparing the values obtained from pH-metric technique.

i) The experimental data of pK and LogK values of various systems are used to understand the effect of substituting groups on proton-ligand and metal-ligand stability constants and to test the validity of Log K = (a pK + b) relation.

ii) One of the systems at different ionic strengths has been carried out. The values...
of proton-ligand and metal-ligand stability constants at different ionic strengths are utilised to estimate the thermodynamic stability constants at zero ionic strength and to know the exact nature of complexation equilibria.

iii) To understand the role played by the solvent medium on the stability constants of the metal complexes, $\log K$ values for some systems are evaluated in different percentage of dioxane-water and ethanol-water mixtures. The data of $pK$ and $\log K$ values are utilised to examine the relation between $pK$ and $\log K$ vs ($\frac{1}{D}$) & molefraction of dioxane.

iv) One of the systems of peptide complexes is studied at different temperatures for determining the thermodynamic parameters such as enthalpy, entropy and free energy.

v) The parameters such as apparent molal adiabatic compressibility, $\phi^{\text{ad}}$, apparent molal volume $\phi_v$, excess volume $V^e$ have also been determined and the values are used to discuss the ionic interactions.

vi) Ternary complexes of some peptides with Ce(III), Nd(III) and Th(IV) metal ions have been investigated for determining the metal-ligand stability constants and

vii) Biological applications of binary and ternary complexes of peptides with Co(II), Cr(III), Ce(III) & Th(IV) metal ions have been investigated to see the effect on germination, survival and seedling height of Wheat & Barely plants.

(vi) and (vii) are discussed in separate chapters.

Following peptides are used as chelating agents (ligands):

1) Glycyl-Glycyl-Glycine (L1)
$H_2N\text{CH}_2(\text{CO N\text{H CH}_2})_2\text{COOH}$

2) L-Alanyl-L-Alanyl-L-Alanine (L2)
$\text{CH}_3\text{CH}(\text{NH}_2)(\text{CO N\text{H CH(CH}_3})_2\text{COOH}$

3) DL-Alanyl-DL-Phenyl Alanine (L3)
$C_6\text{H}_5\text{CH}_2\text{CH}\{\text{NCOCH(NH}_2)\text{CH}_3\}\text{COOH}$

4) DL-Alanyl Glycine (L4)
$\text{CH}_3\text{CH(NH}_2)\text{CONH CH}_2\text{COOH}$

5) DL-Ananyl-DL-Alanine (L5)
$\text{CH}_3\text{CH(NH}_2)\text{CONHCH(CH}_2)\text{COOH}$
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