CHAPTER I

INTRODUCTION

In ancient times, metals have been used to treat many diseases in empirical fashion. Until the landmark discovery of cis-platin, inorganic chemists were never aware of the use of metal complexes as anticancer agents. The success of cis-platin has revolutionised the medicinal inorganic chemistry.

1.1. METAL COMPLEXES IN MEDICINE

It is well known that metals play significant roles in human biology. They control the structure and function of many enzymes and metabolism in the human body. Either their deficiency or over accumulation is the origin of many biological problems. So metals are called as “two-edged weapons”. For example, iron deficiency causes anemia whereas high level of iron in the blood stream is responsible for most of the human diseases from Alzheimer’s to malaria. Therefore, determination of dose level of metals is important in medicinal applications. Since biological functions lie on the nature of metals and their oxidation state, utilizing their properties at pertinent level for human diseases is an active area of research in bioinorganic chemistry. Basically, metal complexes show unique features like variable redox states, wide range of coordination number, geometries etc., these cannot be accessible in organic molecules. For example organic molecules exhibit geometries range from linear to trigonal and planar to tetrahedral. Whereas, metal complexes can generate numerous geometries like linear, trigonal planar, tetrahedral, octahedral and many others.
These kinds of structural diversity provide opportunity to design the molecules for better bimolecular interactions. Moreover, many organic compounds used in medicine do not have a purely organic mode of action. Some are activated or biotransformed by metal ions, including metallo-enzymes, others have a direct or indirect effect on metal ion metabolism. Hence metals are best scaffold to construct therapeutic molecules.

The metal ions and metal complexes interact with bio-macromolecules such as DNA (deoxyribonucleic acid), RNA (ribonucleic acid), enzymes and proteins. So studies based on the interaction of metal complexes with bio-macromolecules are essential for pharmacological activities. The binding affinity between the complex and the bio-molecule can be tuned by the choice of the metal and ligands. Hence, this platform prompted many researchers to show their interest on metal complex based diagnostic and therapeutic agents.

1.2. BIOLOGICAL IMPORTANCE OF COPPER AND COBALT

Both cobalt and copper are essential elements for living organisms. Copper is important for the synthesis of collagen. Collagen is a key molecule responsible for the rigidity, mechanical strength and competence of bone and elasticity of skin. Copper is involved in the synthesis of the skin pigment called melanin. Copper is a basic element for the development of brain and nervous systems. It is also involved in the synthesis of neurotransmitters. It is important for the healthy white blood cells. It acts as a cofactor for an enzyme involved in the coagulation of blood. The blood vessels are surrounded and protected by connective tissue, where copper helps to sustain their elasticity, particularly for the aorta and smaller arteries. Copper plays a significant role in the conversion of Fe(II) to Fe(III) and also helps the transport of iron. Copper-containing enzymes are
essential for the generation of cellular energy inside the mitochondria.\textsuperscript{9} They are used in the neutralisation of harmful free radicals.\textsuperscript{10}

Cobalt, in the form of vitamin B\textsubscript{12} (cobalamin), plays a number of crucial roles in many biological functions such as DNA synthesis, formation of red blood cells, maintenance of nerve tissues, growth and development.\textsuperscript{11} Recent reports suggest that, cobalt is involved in immune processes.\textsuperscript{1} Unbalanced level of cobalt will influence in infections and malignancies. Most importantly cobalt acts as an activating agent for various enzyme systems.\textsuperscript{12} As it involved in the regulation of some definite cellular processes it is called as a biological response modifier.

1.3. BIOLOGICAL IMPORTANCE OF SCHIFF BASE METAL COMPLEXES
Schiff base was named after Hugo Schiff in 1864. It is a compound containing a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group. In a broad sense Schiff bases have the general formula as $\text{R}_1\text{R}_2-\text{C}=\text{N}-\text{R}_3$, where $\text{R}$ is an aryl or alkyl group. In general, Schiff base reactions are widely used in chemistry due to their mild reaction conditions and high reaction rates. For many years, Schiff base complexes are being used as promising candidates in chemical biology because of their unique properties.

The medicinal applications of Schiff bases are still in current interest due to their broad spectrum of action. A variety of metal complexes with different metal centres (mainly d-block elements) and Schiff base ligands of diverse structures were synthesised and studied for their biological activity.
Chapter I

There are many reports which suggest that nature of aldehyde/ketone and amine group will determine the biomolecular interaction and biological applications of Schiff bases.\textsuperscript{13} For example Fe(II) containing Schiff bases derived from various amino acids such as L-alanine, L-phenylalanine, L-aspartic acid, L-histidine and L-arginine and o-hydroxynaphthaldehyde show different antibacterial, antifungal activity.\textsuperscript{14}

In addition to various ligands, metal ion also significantly influences the activity of the Schiff base metal complexes. For example various metals like Cu, Zn and Cd containing same Schiff base ligands derived from 2-acetylpyridine and L-tryptophan have been tested for anticancer activity with MDA-MB-231 breast cancer cells.\textsuperscript{15} In that Cd complex shows higher activity than Cu and Zn complexes. Recently Suna Wang et al. have reported that Cu and Ni Schiff base complexes act as anticancer agents for human lung carcinoma cell line, colon carcinoma cell lines, promyelocytic leukemia cells and the chronic myelogenous leukemia cell line.\textsuperscript{16} Here the nuclearity and metal ions in the complexes notably change the anticancer activity of the complexes. Since Schiff base complexes are vibrant candidates in biopolymer interaction they show promising results in biological applications as antifungal agents, anti bacterial agents, anti cancer agents, anti HIV agents etc. Recently ruthenium(II) and rhodium(III) thiosemicarbazone Schiff base complexes have been reported with antiparasitic activity.\textsuperscript{17} Schiff base complexes, ferrocenyl-L-amino acid copper(II) complexes, iron(III) complexes of pyridoxal Schiff base and thiosemicarbazide iron(III) complexes act as photocytotoxic agents.\textsuperscript{18-20}
Chapter I

1.4. **CANCER**

According to medical dictionary, cancer is not just one disease, but a large group of almost 100 diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death.\(^{21}\)

In human body, for normal biological functions, cells are divided and at one stage cells commit suicide if are they no longer needed. In the case of cancer, cells grow without control.

![Normal Cell Division and Cancer Cell Division](image)

**Fig.1.1.** Pictorial representation of normal and cancer cell division

1.4.1. **CELL CYCLE**

The cell cycle is a process of reproduction of daughter cells by the series of events that take place in a cell. The most important parts of the cell cycle are the
synthesis and duplication of nuclear DNA before division. The different stages in the cell cycle are schematically given in Fig. 1.2.

**Fig.1.2.** The different stages of the cell cycle

**G1 phase:** Metabolic changes prepare the cell for division. Cells are committed to division and ready to move into the S phase.

**S phase:** DNA replication occurs. DNA synthesis replicates the genetic material. Each chromosome now consists of two sister chromatids. Each of the 46 chromosomes is duplicated by the cell.

**G2 phase:** Metabolic changes assemble the cytoplasmic materials for mitosis and cytokinesis.

In this stage, the cell double checks the duplicated chromosomes for error, making any need of repair. The G2 check point did not allow the cells for the next stage if DNA is damaged or not replicated.
M phase: A nuclear division (mitosis) followed by a cell division (cytokinesis). In this phase the M check point verify whether the chromosomes are properly aligned or not. It only allows the properly aligned cells for mitosis.

The separated daughter cells form M phase, enter into quiescent phase, $G_1$ or $G_0$. Then the cells pass through another cycle depending on external conditions. If they remain in $G_1$ phase for prolonged periods of time they arrest as quiescent. Even though the non-dividing cells in a $G_0$ phase are metabolically active they do not actively proliferate. So, external condition is needed to stimulate the $G_0$ cells into $G_1$ phase. At this $G_1$ check point cell death occurs via apoptosis if DNA is damaged.

1.4.2. APOPTOSIS and NECROSIS

In 1972 Kerr and colleagues classified cell death into two categories, one is apoptosis and other is necrosis. Apoptosis is a well controlled and organised individual cell death which occurs in multicellular organisms. This biological event leads to characteristic morphological changes such as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation. Apoptosis is triggered through two signalling pathways namely intrinsic pathway and extrinsic pathway. Intrinsic pathway (mitochondrial pathway) is initiated from within the cell. This pathway is often activated in response to signals resulting from DNA damage, loss of cell-survival factors or other types of severe cell stress. Normally, pro-apoptotic proteins are released from the mitochondria to activate caspase proteases and trigger apoptosis.

The extrinsic pathway begins outside the cell through activation of pro-apoptotic receptors on the cell surface. These are activated by molecules known as pro-apoptotic ligands.
Necrosis is the name given to accidental death of cells. Upon compared with apoptosis, clean up of necrotic cell debris by phagocytes of the immune system is generally more difficult, as the disorderly death generally does not send cell signals which tell nearby phagocytes to engulf the dying cell. This lack of signalling makes it harder for the immune system to locate and recycle dead cells which have died through necrosis than if the cell had undergone apoptosis. The release of intracellular content after cellular membrane damage is the cause of inflammation in necrosis.

Fig.1.3. The two different type of cell death
Apoptosis does not cause any inflammation. So impair the cell proliferation through apoptosis is an effective approach in chemotherapy. Metal complexes interact with DNA in different mechanism. Thus DNA interactions lead to the cell death via apoptosis. So in anticancer study, it is important to study the type of cell death while screening the potential of the metal complex for chemotherapy.

1.4.3. NEED FOR EFFECTIVE CANCER DRUG

Cancer is one of the most onerous health problems harassing people worldwide. Based on WHO report 14 million new cases and 8.2 million cancer related deaths in 2012 and by 2030 there will be 21.4 million new cases diagnosed every year.22 Among many types of cancers, breast cancer is the most common cancer in women worldwide. In 2012, nearly 1.7 and 1 million new cases diagnosed with breast and lung cancer respectively.

In clinics, various treatments such as surgery, chemotherapy, radiation therapy, hormone therapy, immunotherapy, hyperthermia and photodynamic therapy have been used to treat the tumors. Nevertheless, the type and the combination of treatments have been decided based on the type and stage of the cancer.

In many cases, the cancer is not diagnosed at its early stage. So most of the patients are along with other treatments they are subjected to chemotherapy. The metal based chemothrapeutic drug cis-platin gives almost 90 % curable rates in bladder, small cell lung, head and neck cancers. But the dark side of the platinum based drugs are neurotoxicity, hepatotoxicity, nephrotoxicity and the inherent cell resistance.23 These are the biggest problems in the clinical applications. It alerts the need for effective non-platinum based metal complexes. There are several metal complexes which are reported with anticancer activity which show better
activity than *cis*-platin and its derivatives. But only a very few compounds have come to market and few of them in clinical trials. Besides, in chemotherapeutic drug designing, reducing the side effects by selectively targeting the cancer cell by blocking their proliferation via bio-molecular interaction is still a challenging one.

Despite active progress in the drug design, currently used anticancer drugs show some severe pharmacological deficiencies like cell specificity, poor water solubility, decreased serum half-life, membrane penetration, drug resistance and excessive systemic toxicity towards other organs. It limits their effectiveness in current clinical practice. It can be overcome by incorporating the drugs into biological or synthetic macromolecules like proteins, graphene, dendrimers, polymers etc. This idea of covalently attaching chemotherapeutic agents to a water-soluble polymer was first proposed by Ringsdorf.

1.5. POLYMER-METAL COMPLEXES AND THEIR IMPORTANCE AS ANTICANCER DRUGS

A polymer-metal complex is nothing but a coordination complex formed between a metal ion and a ligand anchored on a polymer matrix. These polymer-metal complexes can be obtained by the reaction between a ligand of the polymer backbone and a simple metal complex having coordinating ability. Based on the mode of coordination of metal complexes to the polymer chain, these polymer-metal complexes are classified as follows:
Pendant Complexes

Monodentate Pendant Complex

\[ \text{L} + \text{M} \rightarrow \text{E} \rightarrow \text{M} \]

Polydentate Pendant Complex

\[ \text{L L L L L L} + \text{M} \rightarrow \text{M} \]

Intra-polymer chelates

\[ \text{L L L L L L} + \text{M} \rightarrow \text{M} \]

Inter-polymer chelate

\[ \text{L L L L L L} + \text{M} \rightarrow \text{M} \]

Fig. 1.4. Schematic representation of pendant-type polymer metal complex.

1.5.1. Degree of coordination in polymer metal complexes

In the above figure the degree of coordination of the polymer metal complex (x) means the molar ratio, [metal complex] / [repeating unit of polymer ligand]. If all the repeating units in the polymer chain are coordinated to metal, the value of ‘x’ becomes unity.
Grafting of drugs into the polymers have started nearly 60 years ago. Initially, poly(vinylpyrrolidone) (PVP) and dextran based materials were used for dressing wounds. After the clear concept on polymeric drug conjugate from Helmut Ringsdorf, polymer therapeutics became an emerging area in chemotherapy. In 1994, first synthetic polymer–drug conjugate, N-(2-hydroxypropyl) methacrylamide (HPMA)-doxorubicin entered into phase I/II clinical trials. After that many polymer drug conjugates based on HPMA copolymer–doxorubicin (DOX), HPMA copolymer–DOX–galactosamine HPMA copolymer–camptothecin, HPMA copolymer–paclitaxel and HPMA copolymer –platinates were reported.

Utilizing the advantage of polymeric materials, cis-platin and oxaliplatin chemotherapeutic drugs anchored on HPMA (hydroxypropylmethacrylamide) polymer which later patented as AP5280 and ProLindac™ respectively were discovered. Both these compounds show improved anticancer activity than parent platinum complexes. Currently ProLindac™ is in phase II clinical trials.

Based on the success of polymeric metal complexes many metal complexes anchored on various polymers and their invitro cytotoxicity towards different cancer cell lines were reported. However clinical applications of them are yet to be proved.
In polymer drug conjugates, the promising therapeutic value not only depends on the metal complex but choice of the polymer is also very important for the biological applications.

1.6. POLYETHYLENEIMINE

Polyethyleneimine (PEI) is a cationic polymer which exits in two forms, linear polyethyleneimine (LPEI) or branched polyethyleneimine (BPEI). It has the repeating unit of one amine group and ethylene group. In LPEI, all the amine groups are secondary amines. In contrast, BPEI has primary, secondary and tertiary amine groups in the ratio of 1:2:1. Some of the reports suggest that it is used in drug delivery and cell imaging. It is a water soluble polymer and anchoring the insoluble metallo drugs will improve the metal complex activity in nucleic acid and serum protein binding. In addition to that BPEI has increased the cancer cell uptake of metal complexes.

Fig.1.5. Schematic representation of polyethyleneimine
Polyethyleneimine has unique properties which makes it an interesting candidate in various areas. Few of the notable properties are given below

- Good water solubility provided by the hydrophilic amine groups, and anchoring of metal complex in it increases the water soluble nature of the metal complexes;
- High capacity for metal uptake due to high local concentration of functional groups;
- High molecular weight, which allows an easy separation, by the usual methods (e.g. membrane filtration) of polymer metal complexes from low molecular weight species present in solution.
- Good chemical stability; Reversible complexation is easily achieved for labile metal ions with acids and strong ligands.
- High flexibility of the molecular conformation enables it to achieve an optimum configuration for complex formation.
- The behaviour of aqueous solutions of the polymer molecule is largely determined by the combined action of the inert part of the molecule causing hydrophobic interactions and of the polar amine groups causing hydrophilic interactions with the solvent.

1.7. DNA AND ITS INTERACTION WITH METAL COMPLEXES

DNA is the fundamental bio-molecule for human life and its replication is a key step in cell proliferation. If DNA is mutated by any reason that will lead to uncontrolled cell division thus causing cancer. So, blocking the cell division through DNA – complex interaction is foremost strategy in chemotherapy. The structure and the composition of DNA offer many target cites for metal complexes. DNA is a long polymer made up of nucleotides. It contains the genetic instructions
which are essential for organism to develop, live and reproduce. Each nucleotide contains a phosphate group, a sugar group and four nitrogen bases namely adenine (A), thymine (T), guanine (G) and cytosine (C). (Fig. 1.6)

Fig. 1.6. Structure of nitrogen bases and their link in DNA.

Nuclear DNAs are located in the cell nucleus and small amount of DNA is found in the mitochondria, called as mitochondrial DNA.

In DNA, the nucleotides are attached together by hydrogen bonds and form two long strands that spiral to create a structure called a double helix. DNA strands are closer together on one side of the helix than on the other. When the backbones are far apart and close together they produce major and minor groove respectively. (Fig. 1.7)
Fig. 1.7. Types of Groove present in DNA double helix.

In general, metal complexes interact with DNA either in covalent and/or non-covalent fashion.

Fig. 1.8. *Cis*-platin and guanine base intrastrand cross-link
The best example for the covalent interaction is the adduct formation between cis-platin and DNA (Fig. 1.8). Cis-platin forms intrastrand cross-link with guanine base in DNA at the N7 position. This adduct is found in a way that the DNA duplex is bent around 40° which leads to inhibition of its DNA's functions.

Non-covalent interactions such as intercalation, groove binding and electrostatic attraction depends on the various factors like nature and size of the ligands and metals, oxidation state of the metal and geometry of the metal complexes etc.

![Fig. 1.9. Schematic representation of intercalation between DNA and metal complex](image)

Intercalation is the process in which planar aromatic ligands insert between the two adjacent DNA base pairs (Fig. 1.9). In order to accommodate the ligand, DNA will undergo vertical separation between the base pairs, which increases the cavity for the incoming ligand and it is stabilised by π–π* stacking between the base pairs and aromatic ring system, leading to lengthening, stiffening and unwinding of the DNA helix.
The nature of the helix structure provides groove binding to small molecules and metal complexes (Fig. 1.10). Based on its own size and shape, the metal complex fits into the grooves, with displacement of water. The groove binding is facilitated by van der Waals interactions between the grooves and the molecules. It results in the distortion of the DNA conformation.

**Fig. 1.10.** Schematic representation of groove binding between DNA and organic molecule

The electrostatic interaction arises between the positively charged molecules (metal complexes) and the negatively charged phosphate backbone of DNA chain. Electrostatic interaction is the force between the positive charge of the metal complex and negative charge of the base pair of the DNA and phosphate back bone (Fig. 1.11).
Chapter I

This kind of interaction is generally weak under physiological conditions. Compared to covalent interactions non-covalent interactions are better one in DNA targeted chemotherapy.

1.8. SERUM ALBUMINS AND THEIR IMPORTANCE

Serum albumins are the most extensively studied proteins because of their efficient role in drug delivery and remarkable binding properties with metal complexes. They make important effects on the distribution, absorption, free concentration and metabolism of the drugs. Many endogenous and exogenous compounds such as drugs, hormones, xenobiotics and fatty acids are entered into the blood stream, are transported and forming a complex with serum albumins. They contribute to the colloid blood osmotic pressure and the maintenance of blood pH.

1.8.1. BOVINE SERUM ALBUMIN (BSA)

BSA molecule is made up of three homologous domains (I, II, III) that are divided into nine loops (L1-L9) by 17 disulfide bonds. It consists of 585 amino acids in a single polypeptide chain cross-linked with disulfide bonds. BSA has two
tryptophan moieties (Trp 134 and Trp 212) which are responsible for the intrinsic fluorescence (Fig. 1.12). Trp -134 is located on the surface of the molecule and Trp-212 is located within a hydrophobic binding pocket of the protein. Each domain is composed of two sub-domains (A and B). Bovine serum albumin (BSA) has been one of the most extensively studied proteins, especially of its structural homology with human serum albumin (HSA).

![Three dimensional structure of bovine serum albumin with tryptophan residues are in green colour](image)

**Fig. 1.12.** Three dimensional structure of bovine serum albumin with tryptophan residues are in green colour

### 1.8.2. METAL COMPLEX-SERUM ALBUMINS INTERACTION

In drug administration, the metallo-drug affinity for plasma proteins directly influences the metallo-drug concentration in the bloodstream and its biological effect. The effect of weak protein binding leads to short lifetime or poor distribution, while strong binding leads to a decrease in drug concentration. As it is the unbound fraction of the metallo drug that shows pharmacological activity, studies on interaction of metal complexes with serum proteins are important in the field of pharmacokinetics, pharmacodynamics and medicinal chemistry.
Binding to plasma proteins is recognized as a crucial step in accessing the bioavailability of metal complexes. That’s why, drug binding ability with serum protein binding is essential FDA requirement in screening potential therapeutic agents.

Among the serum albumins, bovine serum albumin (BSA) is an attractive macromolecule because of its availability, low cost and unusual ligand binding properties. So, studies on the binding of drugs with protein will help to interpret the metabolism and transporting process of metallo-drugs and also to understand the relationship between the structure and functions of proteins.
1.9. REFERENCES


22

23