Synthesis, Characterization and Biological Activities of Mixed Ligands Copper(II) 4-(4-nitrophenyl)piperazine-1-carbodithioates

Islamabad

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Master of Philosophy

in

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by

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DECLARATION

This is to certify that this dissertation entitled "***Synthesis, Characterization and Biological Activities of Mixed Ligands Copper(II) 4-(4-nitrophenyl)piperazine-1-carbodithioates***" submitted by ***Mr. Sabih-Ud-Din*** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan, as satisfying the dissertation requirements for the degree of ***Master of Philosophy in Inorganic/Analytical Chemistry***.

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IN THE NAME OF ALLAH

THE COMPASSIONATE

THE MERCIFUL

Dedicated to

My Parents

“Allah will exalt those who believe among you, and those have knowledge to high ranks”

(Al-Quran)

Sayings of Holy Prophet (S.A.W.)

“If anybody goes on his way in search of knowledge, Allah Almighty will make easy for him the way to paradise”

(Sahih Muslim)

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ABSTRACT

Three new copper(II) compounds, [Cu(2,2′-bipyridyl)ClL] (**1**), [Cu(1,10-phenanthroline)ClL] (**2**) and [Cu(ethylenediamine)ClL] (**3**), where L = 4-(4-nitrophenyl)piperazine-1-carbodithioates, have been synthesized and were characterized by elemental analysis, FT-IR, UV-Visible spectroscopy and cyclic voltammetry. Spectroscopic analysis confirmed the monodentate behavior of ligand (L) and a square-planar geometry around Cu atom. The cyclic voltammetric results (CV) revealed that the redox behavior of the complex **1**, is highly pH dependent. A subsequent DNA interaction of complexes **1**and **2** by CV proved them to be good DNA binders. In addition to this, complexes **1** and **2** have shown the scavenging activity against DPPH free radicals, and the former complex is a head in this race than latter as evident from their IC_{50} values.

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INTRODUCTION

1.1. Carbamates and Their Importance

In 1950s researchers show great interests in the Carbamates, due to their similar mode of action to anticholinesterase on Central nervous system as that of organic phosphate exert on pests. Their mode of action is similar to that of organophosphate but different in action as inhibitory effect on cholinesterase enzymes as their reversal effect is so rapid that it is difficult to measure the cholinesterase level in the blood, seems to be imprecise or looks as normal. The carbamates are the organic compounds which are derived from carbamic acid (NH_2COOH). The functional groups such as carbamate group, carbamate ester and carbamic acids which have structurally close resemblance with each other and also have the capability of inter-converted chemically with one another. The carbamate esters are also known as Urethanes, the general formula of these carbamates are given below, where R is an alcohol, oxime or phenol and R1 is representing a hydrogen or a methyl group [1] as shown;

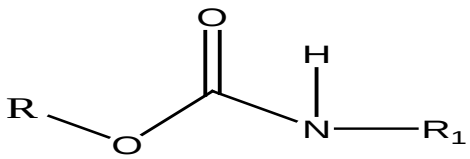


Fig. 1.1: General formula of Carbamate

Carbamates are generally unstable, which are decomposed on exposure to the open atmosphere within few minutes or some sample takes weeks or months. They show great variations in mammalian toxicity, a broad spectrum in their activity and in their perseverance etc [1].

1.2. How Carbamates are Used?

Carbamates are generally used in the form of surface sprays such spray on crops or baits for the control of household pest such as to kill cockroaches, ants, fleas, crickets, aphids, scale, whitefly, lace bugs and mealy bugs etc, as carbamates affect their brains and nervous systems as they inhibit the cholinesterase enzymes. They must apply at low level at home or yards. Even some carbamates also use to control mosquitoes. Sometime some carbamates are found in groundwater at such a high level which is sufficient for its toxic effect for other living systems [2].

In 1956, for the first time the Carbaryl, which is the first successful carbamate, was widely used as a successful insecticide on the basis of its two basic possessing distinct qualities, which has made carbaryl successful for this purpose. The first basic quality is that, it has a very low mammalian toxicity either may take orally or through skin. The second basic quality is, this carbaryl can apply as insecticide to control a wide range of insects. This is the reason that now a days it has broadly use as a lawn and house garden effective insecticide, e.g, Propoxur is highly effective against cockroaches while these cockroaches offer a great resistances to the organophosphate which was used against them, similarly the Bendiocarb, Methomyl and other have also their successful uses [1].

1.3. Side Effects of Carbamates

Now a days Carbamates are widely used as insecticides either in the form of dusts or sprays. These carbamates are very crucial for our society but they also exert some side effects as they entered into human body either through the skin though cuts or scratches directly or by foods and drinking water contaminated with carbamates or through breathing the cabamates. The toxicity caused by these carbamates are very similar to that of organophosphate but the persistence of these carbamates are very low and very rapidly recovery occurs, which is the advantage over organophosphate and even on the exposure required no medical treatment for their recovery within 4 hours and without any medical treatment [1,3].

If a person exposed to these carbamates either accidently or by forced, he may suffers as headache, light headedness or dizziness, also slow heart beats, weakness, excessive salvation, nausea, muscle fasciculations, difficulties in breathing, vomiting, also suffers from sensation of swelling or tightness in chest and excessive salivation. Eyes are also largely effected by these carbamates and their infections result in uneasiness in eye muscles which result in blurred or dark vision and also effect on pupils to change their size which result in far reaching tearing and redness of eyes. While contact of carbamate for long time, can cause loss of weight, appetite, weakness and generally feeling sickness [1,2,4,5].

If the condition observed is more severe then the effected one must be treated medically. Generally Carbamates are not accumulating in the body and their persistence is also low, that is why these are not considered as cancer causing agent, but exception is

also there e.g Carbaryl (SEVIN) and carbofuran (FURADAN) , when these are combined with some other elements in the stomach, then there a possibility that these are considered as cancerous chemicals. There are also some carbamates such as carbofuran (FURDAN), have some role on birth defects or caused reproductive problems in the testing animals. There are no definite health effects from long term exposure nor do they have longer persistence in the body that is why these carbamates have no role in causing delayed neurotoxicity as usually observed for organophosphate. So from above studies, the Carbamates are not considered as mutagenic, carcinogenic or teratogenic substances [1,4,3,6].

1.4. Treatment of Poisoning

Although carbamates are not so highly toxic but if it is entered in the body through eating food or drinking contaminated water, then vomiting must be tried similarly for further prevention, the dirty cloths should be discarded and the whole body should be washed with soap and water so that the chances for entering through cuts and scratches be minimum. But this is also happened that although these carbamates are relatively non-toxic but if a small amount of these carbamates got entered into the body of a person through some means and if that person has already organophosphates in the body but who does not give some clinical symptoms upto now, then these carbamates may give some conditions of acute toxicity, and this true because about 70% hopelessness in activity for cholinesterase enzymes are carried out with time without noting any clinical symptoms, but if further decrease in cholinesterase activity occurs then this situation may lead to acute toxicity/poisoning which may cause due to precipitation of these carbamates with some other external chemicals. And if decrease in the activity of cholinesterase enzymes carry for longer than 48 hours, then this means that this decrease may be due to the carbamates [1,5,6].

1.5. Sulfur Analogue

The general formula of carbamate is $ROC(=O)NR_2$, shows that the carbamate has two oxygen atoms per unit structural formula , and there is a possibility that either one or both of oxygen atoms can be replaced by sulfur atoms.

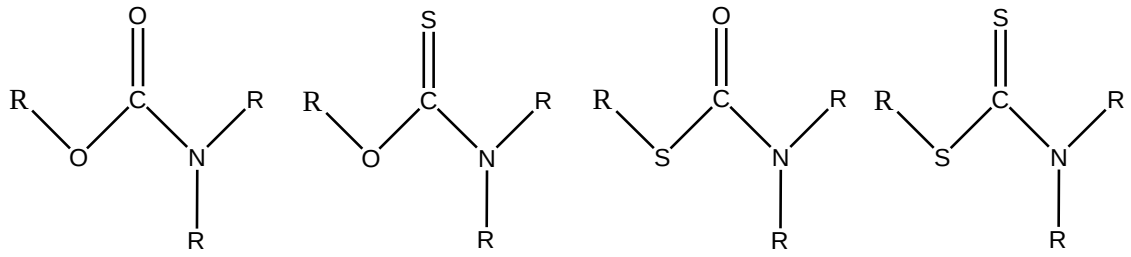
1.5.1. Thiocarbamates

The similar structure obtained by replacing only one oxygen atom in carbamate structure by sulfur atom is called as thiocarbamates. The thiocarbamates have usually two structural isomers, the *O*-thiocarbamates and *S*-thiocarbamates.

1.5.2. *O*-thiocarbamates, $ROC(=S)NR_2$ (2), in which the carbonyl group (C=O) is replaced by a thiocarbonyl group (C=S)

1.5.3. *S*-thiocarbamates, $RSC(=O)NR_2$ (3), in which the R–O– group is replaced by a R–S– group

These isomers are interconverted to each other as *O*-thiocarbamates have the ability to isomerise to *S*-thiocarbamates, through the Newman Kwart rearrangement.



1. Carbamate 2. O-thiocrbamate 3. S-thiocarbamate 4. Dithiocarbamate

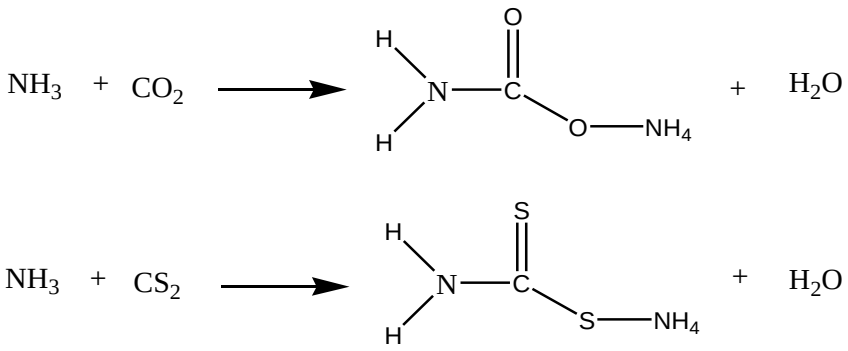
Fig: 1.2: Carbamate and its Analogue

The Sulphur containing ligands such as cysteine and glutathione, show a very strong attraction for transition metals e,g. Palladium and Platinum to make very stable Pd(II) and Pt(II) complexes. There is always a competition develop between these Sulphur containing ligands and DNA for any drug which has been used as an antitumor agent and this competition provides a great biological importance for the calculation of equilibrium constant for the replacement of externally modified ligands such as insoline, glycine, or methionine by these cystin and glutathione, this equilibrium constant give us an idea about antitumor agent that how much these ligands effectively modify the drug [7].

There are large number of organic molecules that are used as organic ligands for complexing various metals, examples of N, S containing donar ligands are Bipyridine, 8-hydroxy quinolone, 4-hydroxy benzothiazole, PAN,*O*-phenanthroline, Picolinic acid etc. these contain the most active atoms which results in chelation of the metals.

1.6. Dithiocarbamates

The carbamates which are derived on replacing both oxygen atoms by sulfur atoms in unit structure are called as dithiocarbamate , $RSC(=S)NR_2$. The carbamates are generally the half amides of carbonic acids, while the product dithiocarbamates obtained on replacing oxygen by sulfur, are the half amides of dithiocarbonic acids, which are generally represented by simple equations as;



Scheme.1.1: Ammonium salts of dithiocarbamates

A dithiocarbamate is a broadly used as functional group in organic chemistry. It is also used as a common ligand in inorganic chemistry e.g. Sodium diethyldithiocarbamate. This dithiocabamate can be synthesized on reaction with most of the primary and secondary amines with carbon disulfide and sodium hydroxide [8]. Dithiocarbamates are more interesting ligand among other ligands as DTCs contain S and N donar atoms in one structure, have more resemblance with metalloproteins as most of them contain both S and N donar atoms [9]. They are basically used as ligands to chelate the metal ions so that to increase the specificity of metal complex for their actions. These Dithiocarbamates readily react with many metal salts such as copper, ferrous, ferric, cobaltous, and nickel salts and mostly results in octahedral geometry for these metals.

The dithiocarbamates which are the semi-amides of dithiocarbonic acid, there 1,1-dithio monoionic form can be represented by four resonance structures, but more

contribution from the structure ‘D’ for dithiocarbamate which is basically supported by the band appears at 1480-1550 cm^{-1} which is assigned to v(C=N) [10,11,12].

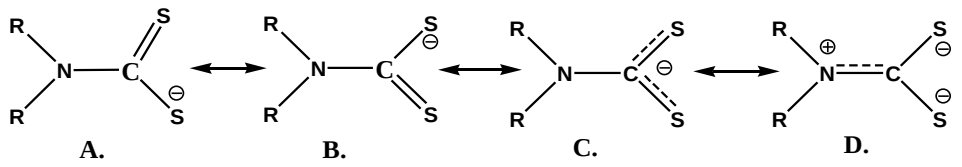
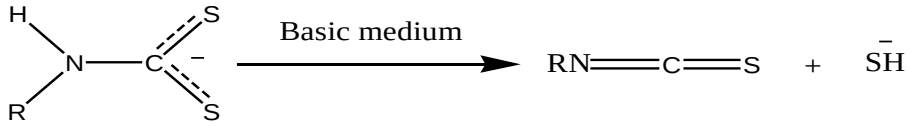
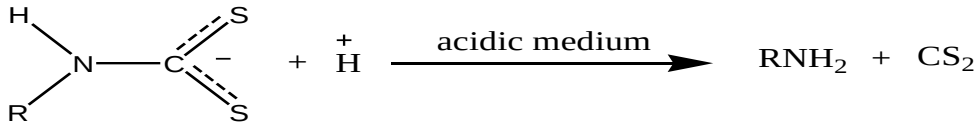


Fig. 1.3: Resonance (canonical) forms of dithiocarbamate

Dithiocarbamates are usually stable in basic and neutral conditions but those which are obtained from primary amines, are easily decomposed to isothiocyanates under the basic conditions as shown in scheme (A) while most of the dithiocarbamates are decomposed to a amine and CS_2 under acidic conditions as shown in scheme (B) [10,13,14,15].



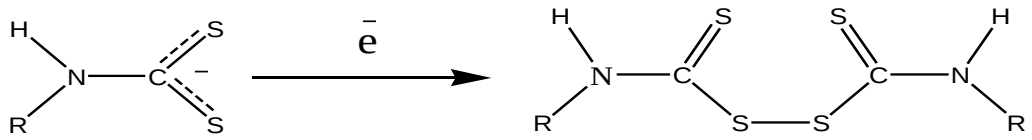
(A)



(B)

Scheme 1.2: Decomposition of dithiocarbamates

Another characteristic feature of dithiocarbamates is that, these DTCs undergo oxidation reaction very easily resulting thiuram disulphide with good yield. Various oxidizing agents such as Iodine, hydrogen peroxide, bromine and potassium ferricyanide can be used for oxidation of DTCs, mostly proceeds through single electron detachment as shown [10,15]..



Scheme 1.3: Oxidation of dithiocarbamates

Basically, in 1942, Gordon for the first time, used these dithiocarbamates such as tetramethyl thiouram disulfide for the control and treatment of scabies which is a dermatophyte [16] and later on, in 1943, the same complex of metal dialkyl dithiocarbamates were used as a prospective fungicide in agriculture field [17].

1.6.1. Cu-carbamate and Their Biological Importance

There are different types of ligands used for complexation but the ligands which contain nitrogen, oxygen, sulfur or phosphorus atoms or mixer have the ability to form a variety of zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D) or three-dimensional (3D) structures on coordination with different heavy metals such as dithiocarbamates, dithiazone, dithiols. Therefore, the dithiocarbamate anions can be used as a versatile ligand for obtaining a variety of geometries for complexes as dithiocarbamate containing S donar atom, have the ability to act as monodentate or bidentate or bridging ligand for central metal which is also very helpful in acquiring different types of interacting forces for structural features [18,19]. These dithiocarbamates are versatile chelating ligands with wide applications in industries, biology and agriculture. Upto now, there has been a large number of transition metal complexes in which dithiocarbamates with different substituents, are acting as ligands, have been synthesized. These complexes containing dithiocarbamates, have large applications in agriculture and medicine for various applications like that of dithiocarbamates but with improved properties [20]. As the Dithiocarbamates is a versatile ligand that binds and stabilize a variety of metals in low and high oxidation states. This property of the DTCs can be explained on the basis of their resonance forms, as shown before. The DTCs have the ability to stabilize one to four metals in one ligand. Basically show two types of binding modes with different transition metals, either act as monodentate or bidentate. In bidentate mode both the S atoms are connected with one metal atom form four coordinated ring chelate. This simple chelating bidentate mode on the DTCs is more common and is not necessary for all transition metals in all possible oxidation states. This chelating bidentate binding mode may be symmetrical or unsymmetrical. The unsymmetrical bidentate mode is commonly known as aniso-bidentate [10,21]. In monodentate, the dithioate ligand bond through only one S atom and commonly occur when there is no room for bidentate coordinations. In solution, there is conversion between monodentate and bidentate coordinations. Generally, it is very difficult to differentiate between monodentate and anisobidentate binding modes.

Complexes of the dithiocarbamates with any metal can be generally represented as; [10,21,22,23].

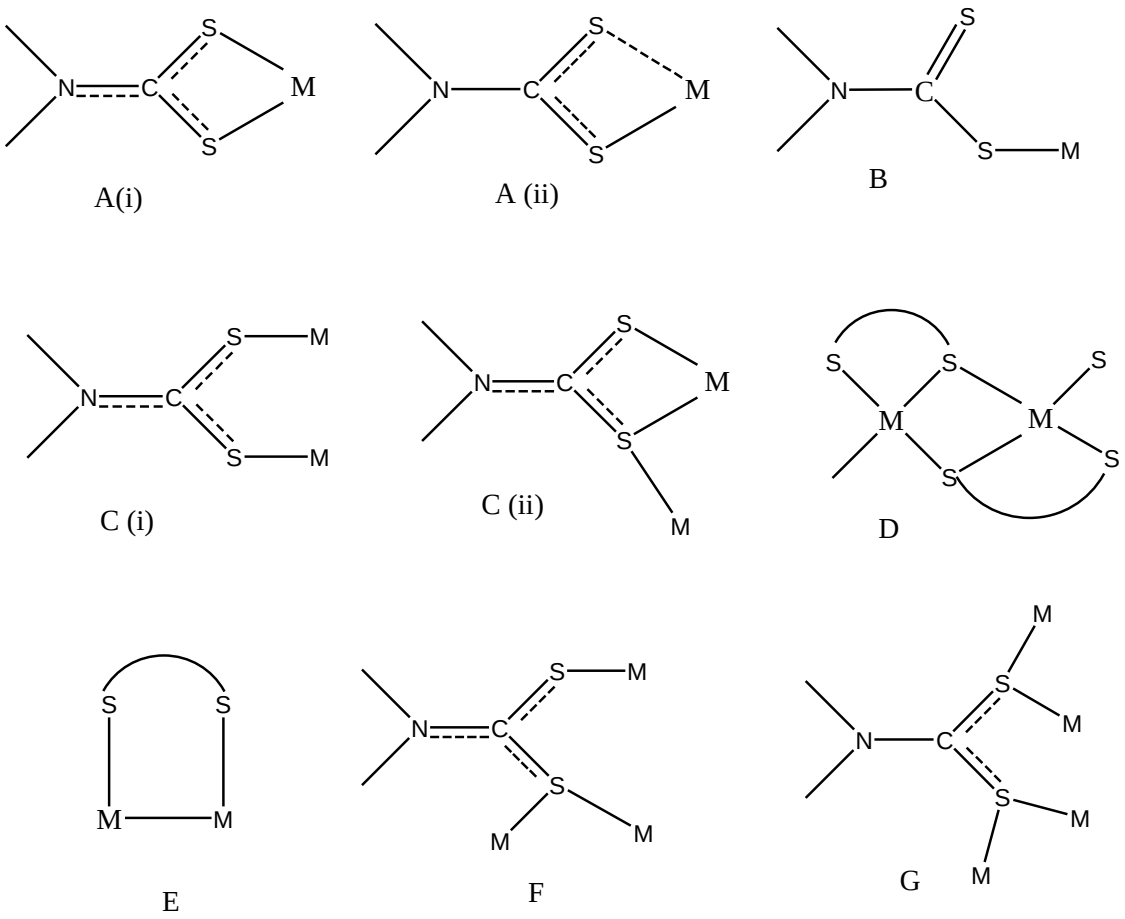
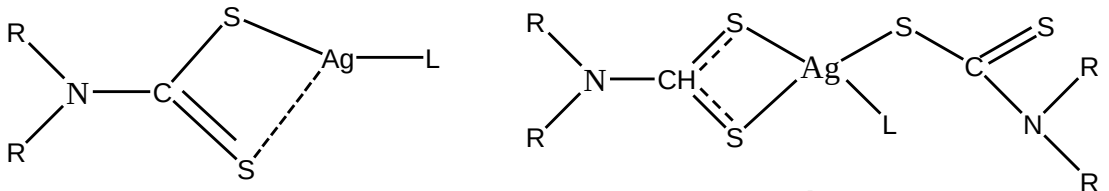


Fig. 1.4: Dithiocarbamate Coordination Modes

In some multiple ligands complexes, some ligand may act as monodentate and some will bound as bidentately with metals, as shown in the figure below [10]



L = different ligand

Fig. 1.5: Anisobidentate and Mixed coordination Modes

So, these dithiocarbamates can accommodate more than one atoms acting as a bridging ligand in a number of ways. The binding mode F and G are rare and limited to the late transition metals [10,24].

The selection of the organic ligands for the synthesis of a biologically active complex, is very important similar to metal selection, because these ligands also have some integral part in biological activities of the complexes [25].

In inorganic chemistry, the dithiocarbamates are used as ligands for multipurposes which have high affinity for many main group elements. These dithiocarbamates have ability to stabilize a wide range of different oxidation states and also to alleviate their coordination geometries, and this is generally observed that even if small changes produced in the ligand structure, results in a noteworthy changes in the complexes by changing their structural behaviors, which are basic requirements for a ligand for designing the complex geometry for biological activities.

In material science and separation science, the complexes of dithiocarbamates with these main group elements and transition metals have wide spacious applications and have also been used widely in the advanced fields as chemotherapeutics, pesticides and as fungicide etc. Although till 1970s no such detail work has been done on these main group dithiocarbamates complexes [26,27,28].

Generally dithiocarbamate complexes of main group elements can be synthesized by the reaction of metal halides with hydrated form of IA metal or the appropriate metal halide with the parent (hydrated) group 1 (I A) metal or ammonium dithiocarbamate salt [29]. In analytical methods, heavy metals are largely identified by using different N-substituted Dithiocarbamates. Also in various materials, traces of copper can be identified and also can be quantitatively measured by using these compounds [26,28].

1.6.2. Structural Features of Dithiocarbamate Complexes

In the complexes, when there is no other ligands are present, then the dithiocarbamate complexes can manage four possible structural geometries as indicate in fig: 8. The square planar coordination geometries (a), the octahedral coordination geometries (b), the four coordinated dimmer (c) and five coordinated dimmer (d) [10].

Mostly the dithiocarbamate complexes are found in square planar and octahedral structural arrangement. Bis(dithiocarbamates) commonly represented as $M(DTC)_2$ where M = Pt(II), Ni(II), Cu(II) etc exhibit the square planar geometries(a) [30]. Tris-

dithiocarbamates for the metals such as $Cu(DTC)_3$ and $Au(DTC)_3$ have structural arrangement in which six sulfur atoms surrounds the central metal atom and acquire the octahedral geometries (b) for the complexes [30]. Complexes of the general types such as $M(DTC)_2X_2$, where X = Cl, Br, I etc, similarly form an Octahedral geometries. The dimmeric forms (c,d) are most commonly found for Cu and Zn metals [30].

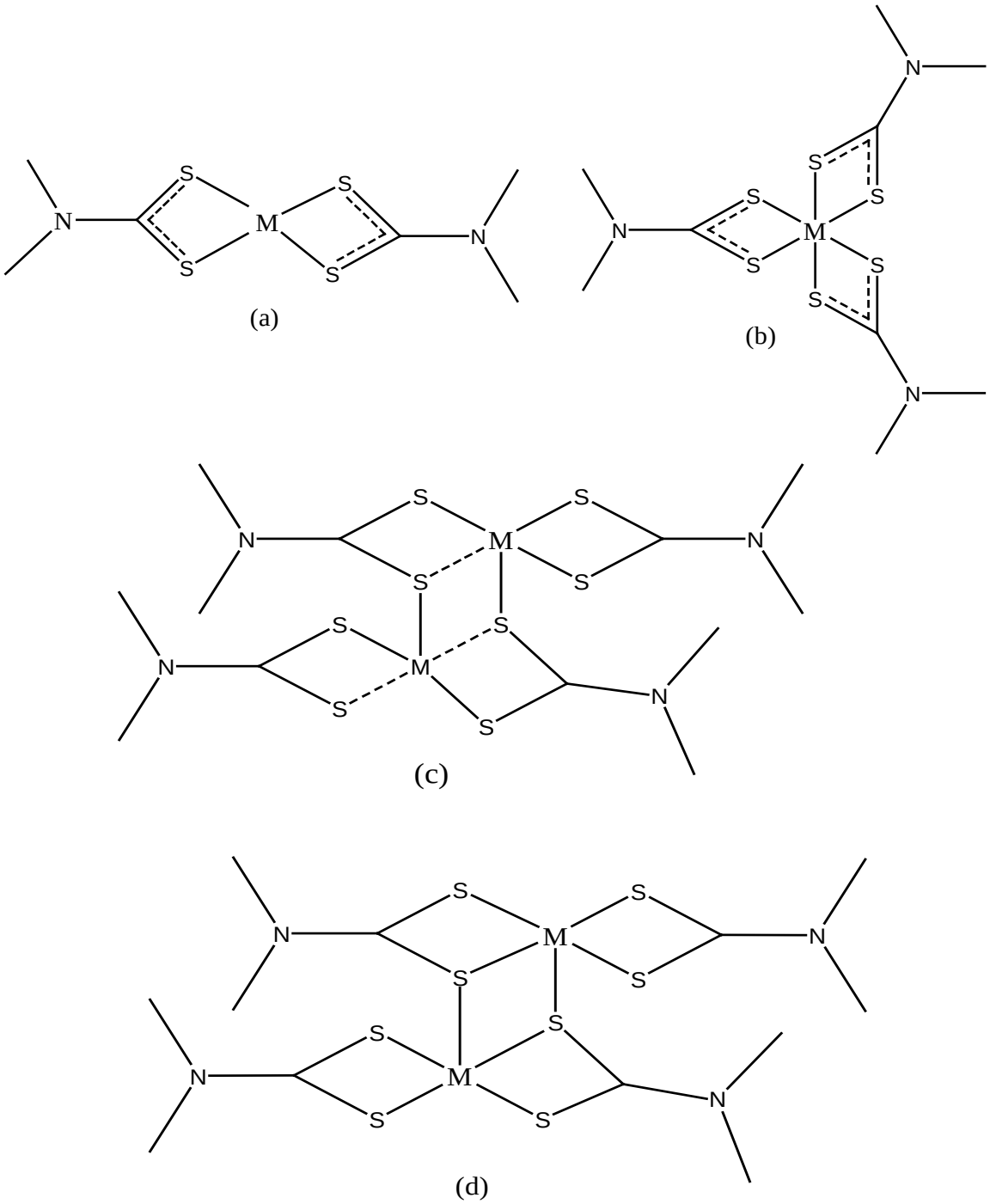


Fig. 1.6: Structural arrangement of dithiocarbamate complexes

From 1978-2003, most of the literature work has been done on studying the structural features of these dithiocarbamate complexes with main group elements.

1.6.3. Characterization Features of the Dithiocarbamates and Their Complexes

Dithiocarbamates show strong absorption bands in the UV-Visible spectrum [31]. Generally three types of bands may be expected to appear in the UV-Visible spectrum which may be due intraligand transitions which are cause by transition from n-π* and π-π* transitions due to the N-C=S and S-C=S functional groups present in the ligand [32]. along these two other transitions might be present for complex form. These may be metal to ligand charge transfer (MLCT) and d-d transitions which are very important for the characterization of complex geometries [10,33].

Infra-red spectroscopy (IR) is extensively used for the characterization of these dithiocarbamate complexes as they generally show some characteristic peaks which are very helpful in the prediction of complex formation and structure elucidation. They exhibit strong absorption band at 1450-1550 cm^{-1} which is due to the ν (C-N) stretching vibrations [10,34]. This band lies between the ν (C-N) band at 1250-1350 cm^{-1} and the ν (C=N) band at 1640-1690 cm^{-1} range.

The stretching vibrations of C-S bond is appeared at the range of 950 cm^{-1} and 1050 cm^{-1}.The coordination of the dithiocarbamate ligand to the central transition metal and their binding modes either act as bidentate or monodentate, can be decided by interpreting the stretching vibration bands appeared for C-S bond in the complexes. The single band in this region shows that dihiocarbamate ligand act as bidentate ligand while splitting of this band by about ± 20 cm^{-1} could be related to monodentate binding mode. Although some investigators have been challenged this criteria for binding modes, but it is widely used for differentiation. The third vibration band which is used for complex formation is appeared in far-IR region in between the 300 and 400 cm^{-1}, which is highly attributed to ν (M-S) vibration band. But this information is not so easy and we should not depend only on these data [34,35,36,37,38,39].

After forming complexes with main group elements, the structural properties of these dithiocarbamates use as ligands, are not considerably changes. Distances (A°) and some angles in the dithiocarbamate structures are generally in the range of as: C--N(R2) ¼ 1.24–1.52 (1.33 mean); 2 Peter J. Heard C--S ¼ 1.52–1.82 (1.72 mean); SCS ¼ 110.1–128.9 (118.6 mean).

The central metal atom is also very important as the SCS bond angle depends largely on size of the central metal atom and it is seemed that this SCS bond angle increases linearly with increasing metal size [29].

1.6.4. Copper Dithiocarbamate Complexes

Dithiocarbamate is a versatile ligand that show a high affinity for transition metals and main group metals, result in a variety of geometries, [10], Copper is one most important metal. Copper (II) show a high tendency towards dithiocarbamates. Most of the Copper (II) bisdithiocarbamates have square planar geometry, due to the fact that crystal field stabilization energy is greater than repulsive energy of the stearic interaction caused by central Cu (II) metal [10], a detail study for these copper complexes provides that copper dithiocarbamate complexes could be built up from simple monomeric units and centrosymmetric dimmeric structures as [40,41]. Cu(II) complexes from simple monomeric units adopt the square planar geometries for central metal as shown, while in dimeric structures each Cu(II) center is five coordinated in which Cu-Cu and Cu-S interactions exist and these arrangement acquire the four and five coordinate geometries for the complexes as shown respectively. Cu (II) complexes are usually paramagnetic and have magnetic moment of 1.6-1.9 BM. [10].

1.6.5. Applications of Dithiocarbamate Complexes

In the past decade, a large number of dithiocarbamate complexes have been synthesized due to their wide applications. Majority of the biomolecules (especially metalloenzymes) contain the transition metal sulphur which may contain one or more bioessential transition metals (molybdenum, iron, copper, zinc, tungsten, vanadium and nickel) [42], and due to presence of M-S systems in dithiocarbamate complexes, they have very close resemblance to that of biological molecules [43]. These dithiocarbamates are used for the treatment of tuberculosis [44] and for cancer [8] and in recent time, their stability has been enhanced for the purpose to be used as for AIDS treatment [45,46]. So by using such type of compounds for these purposes, these compounds must show enough chemical stability in the biological medium for more effectiveness in their action [47].

Most of the drugs that show the anticancer activities, by interacting with DNA in a variety of ways, therefore put forth their effects on biological system, through blocking the replication of DNA and thus causes hindering of the growth of tumor cells. This binding and cleaving the DNA materials provides the basis for drug to design it in a

more efficient form by increasing their usefulness to DNA which depends largely on the binding mode and sympathy towards DNA [48].

There has been so many small molecules which have the capability to bind via covalently or non-covalently or cleave double-stranded DNA at a specific sites as reactive models for protein nucleic acid interactions [49], under physiological conditions that have found wide applications in the field of medicine, molecular biology and biotechnology. Later on the non-covalent interactions got an interesting focus [50], since pioneers have been focused mainly on the interaction of these small molecules with DNA [51]. The different metal complexes that interact with DNA double helical strands, and results in inhibition of transcription and DNA replication, for such type of interactions, there are several specific sites in the DNA molecule: as

(i) between two base pairs (full intercalation),
(ii) in the minor groove,
(iii) in the major groove, and
(iv) on the outside of the helix

The planar organic molecules have the ability to interact with DNA through intercalation, put forward for first time by the Lerman [51].

Most of the complexes containing planar phenanthroline bases, results in the cleavage of the DNA double helical structures. In nucleic acid chemistry, such type of complexes are highly used as foot printing and sequence specific DNA binding agents, also as synthetic models for enzymes inhibitions, as new structural analysis and as therapeutic agents. The drug that interact with DNA, cause cleavage in two ways,

a) Hydrolytic pathway

In which the hydrolysis of the phospodiester bonds take place resulting DNA fragementation which can be rejoined after the removal of drug, this is used for modeling as for the enzymes inhibition.

b) Oxidative pathway

In which the oxidation of sugar moiety or nitrogenous bases take place which is permanent process and is used for foot printing and for therapeutic studies [52].

1.7. Why Transition Metal Complexes Selected for DNA Interaction?

As the metal has the capability to lose electrons from outer shell and thus become electron deficient, which play an important role in biological system, as most of the proteins and DNA and RNA molecules possess electron donar groups so a strong attractive force develop between these which enable these metal ions to interact strongly with the nucleophillic sites on these biological molecules that is requirement for us [53,54,55]. Due to the large variety of structural and electronic properties, the transition metal complexes can be successfully modified for developing as quadruplex DNA binders. These transition metal complexes also have often interesting optical, magnetic and catalytical properties etc that could be transformed for the designing of quadruplex investigation and cleaving agents [56].

These Transition-metal complexes stand out as artificial nucleases, also characterized by high stability, diverse structural features and their unique spectroscopic and redox properties that become the basic interest for the research in the field of medicine and biotechnology. These metal complexes have the capability to bind with the DNA through a variety of interactions and cleave DNA by their intrinsic chemical, electrochemical and photochemical reactivities. In the majority of these complexes, the metal ion acts as a redox centre while the ligands are responsible for the DNA recognitions [50,57].

In the transition metal complexes, a metal has a key role as it occupies the center and has considered as a structural locus which is responsible for organizing the ligands in specific geometries and their relative orientations for optimal quadruplex binding. The geometries of these transition metal complexes can be modified by changing the ligand structure around the metal for retaining the geometry of the complex or by changing the metal at the centre which then result complexes of different geometries, while in organic molecules, to introduce this analogous geometrical changes, are very difficult or impossible in some cases, this behavior is one of the advantage over organic counterpart.

Another advantage of this transition metal is that the electron withdrawing properties of this central metal reduces the electron density of surrounding aromatic ligands which results in electron-poor systems, this behavior results in stronger phai stacking interactions with G-quartets. As the center of the G-quartet is normally occupied by the alkali metal cation, on interaction with the DNA this site is occupied by the

electropositive transition metal by substituting the cationic charge of the alkali metal cation at the center, which result in the electrostatic interaction with the DNA. So by studying the interaction behavior of these libraries of metal complexes with the target DNA with their specific geometry can in turn allow the researchers to establish structure–activity relationships between them.

The current study focuses on designing the transition metal complexes as best quadruplex DNA binders are to use planar molecules. Most DNA binders reported upto now are based on planar organic hetero aromatic systems, which are able to interact through phai stacking with the G-quartets at the ends of a quadruplex, but along with this it is also necessary to demonstrate the structural features of quadruplexes for the designing of these binders. These designed quadruplex DNA binders should not only interact strongly with their target but also exhibit high selectivity for quadruplex versus duplex DNA. For most of the organic molecules which is tested so far, the transition metal complexes have the capability to interact strongly with nucleic acids (including quadruplex DNA) through alternative and/or additional modes, such as direct coordination to bases or the phosphate backbone.

During the past couple of years, there has been so many examples reported that like purely organic heteroaromatic compounds which has been reported as DNA quadruplex binders, the transition metal complexes can also interact strongly and selectively with quadruplex DNA.

Such type of interactions can be studied and confirmed by using a range of experimental techniques. The X-ray crystallography and NMR spectroscopy which is a instrumental technique which is providing structural information about quadruplex DNA in association with small molecules. While the strength of interaction between a given molecule and a quadruplex DNA can be studied by using quadruplexe following spectroscopic and analytic techniques, fluorescence resonance energy transfer (FRET), UV/Vis spectroscopic melting assays, surface plasmon resonance (SPR), fluorescent indicator displacement (FID) assays, mass spectrometry, cyclic voltammetry and dialysis [56].

1.8. Transition Metal Complexes

In field of chemistry, a complex, which is also known as "coordination compound" or "metal complex", which can be defined as a structure which is composed of a central atom or metallic ion or molecules surrounded by molecules or anions which is also known as organic ligands or complexing agent. For a molecule to be act as organic ligand, it must possess a lone pair of electrons which should be easily available to the metal center for the formation of coordinate covalent bonds. These ligands are fixed in a specific direction giving a specific geometry to the complex. Originally, a complex is used for an association of atoms, molecules or ions which are bind with each other through weak chemical bonds that results this association as reversible. While discussing these complexes in coordination chemistry, this reversibility term become a little bit meaningless as most of the metal complexes are formed irreversibly and the bonds responsible for their formation are quite strong.

Nowadays, these coordination compounds have been recognized as useful for constructing molecular information processing systems, especially for biological self-organizing processes nuclease activities. So this is the reason most efforts has been done by studying the synthetic procedures and characterization their structural and chemical properties of the copper complexes as fruitful to be metalloenzyme [58,59].

The organic ligands which are surrounding the metal ion in the open space in a specific manner and the complex that is obtained in this way, possesses a very high thermodynamic as well as kinetic stability. As these complexes possessing a variety of some crucial properties such as usually they are strong, hard, having high boiling point and melting point, also they show magnetism, catalytic and stereo-specificity etc which are responsible for using them in many fields such as contract agents in magnetic resonance imaging or for labeling of biomolecules by using the radioisotopes of the meta for the diagnostic and therapeutic purposes, such as cis-platin which are used as a drug for cancer treatment. By studying their applications in various fields, the metal ion at the center of the complexes must be strongly coordinated with the bifunctional ligands, so that by using these complexes e.g for medical treatment they result no deposition of some harmful radioisotopes in the body while on other side, the conjugation of these complexes through another group to a biomolecule, may become possible [60].

The interactions of these complexes with DNA depend largely on their geometries which are obtained by arranging these organic ligands around the central metal atom. So the structure of the ligands and their arrangement around the central metal atom decide their geometries which have direct effect on the interaction with DNA. In addition, DNA may also grant an important role in binding at a specific site for a specific enantiomeric form of the given metal complexes [51].

1.9. Effect of Complexation on Biological Activities

The metallo elements, which are distributed in the biological systems in a trace and ultra trace quantity but these play a very important role for maintaining the normal biological functions at molecular levels. Although these metallo elements exist at very low level even less than 10 grams in the total in a healthy and adult body, but their existing is very important for maintaining normal body functions in more than this calculated amount, such as for proper functioning of enzymes. On the other hand, if their concentrations exceeds through a specific level, which we called as ambient level, than these metallo elements also play a toxic role on biological functions.

To show the biological activities, these bioelements must be able to form complexes with specific bioligands while there kinetic and thermodynamic properties will decide their biological activities and mode of action for these complexes with DNA. The drug action may be increased by improving their effective penetration to the specific site and this penetration through the membrane will be increased by improving their liphophilcity through the formation of chelates and the more important for a drug to be more specific in its action can be enhanced by using specific ligands for designing it.

1.10. Drug

Generally a drug can be defined as any substance which can be introduced into the body of a living organism generally speaking, is any substance that can be defined as absorbed into the body of a living organism and results in altering the normal functioning in any living organism.

While in pharmacology, the drug can be explained as "a chemical substance which is used for the treatment, curing, prevention, or diagnosis of disease or to improve other good physical or mental qualities or behaviors that are required in the society. These drugs play an important role in biological systems. While looking to their requirements toxic features, these drugs may be advised for a limited duration, or can be

arranged as a regular basis for chronic disorders. but these drugs can be differentiated from endogenous biochemicals as Drugs that are introduced inside the biological systems from outside through some means while the endogeneous chemicals which are also known as hormones, are produced inside the body by some specific organs called as Glands, this can be explained from the example as ,insulin is a hormone when it is synthesized by pancrease inside the body and this insulin is considered as drugs when it is introduced from outside into the body.

There are different types of bio-ligands for example bases that are used as source for nitrogen such as different derivatives of pyridines, pyrimidines and pyrroles, amines such as histamines, carbohydrates such as pentose, glucose and different types of vitamins such as ascorbic acid are known as standards for different biometals.

1.11. Copper Chemistry and Biology

In the field of medicinal chemistry, more interest was focused on non-platinum based anti-cancer agents, for discovering more transition metal complexes with less side effects and which show similar or even better cytotoxic activities. Thus a large number of different complexes containing different transition metals such as titanium, gallium, germanium, palladium, gold, cobalt, tin, ruthenium and copper, have been studied for this purposes. Beyond this, the copper(II) containing complexes has been proved a very interesting one for anticancer therapy, and this idea was taken from a large number of research articles which has been explained in detail the synthesis and cytotoxicity of various copper(II) complexes [25].

The aqueous solution coordination chemistry of one of the nutrientially essential transition metal copper, being among the most abundant element in human body although toxic to human body in large amount, is limited to its three accessible oxidation states (I-III) [61,62,63], copper in the zero oxidation state has an electron configuration of $[Ar]4s^2 4p^6 3d^9$. This copper exists in three different oxidation states as Cu(I), Cu(II) and Cu(III) in their complexes.

Cu(III) Chemistry: Cu(III) form is relatively rare and is difficult to obtain without the use of strong π-donating ligands. This form is very easily reduced, which is generally known as uncommon. These complexes retain a square planar geometry due to the involvement of d^8 Cu (III) electronic configuration. But recently this form also got some attention due to its importance because of its involvement in some biological

processes, based on their geometry [64]. K_3CuF_6 is found as high spin Cu (III) complexes, while the rest of all are found as low spin diamagnetic [65].

Cu(I) Chemistry: The second form Copper(I) which is the lowest oxidation state, has d 10 electronic configuration as d^{10} system shows no Jahn-Teller distortion means forms complexes without any crystal field stabilization energy(CFSE). The copper (I) salts in aqueous medium disproportionate into Cu^{+2}(aq) and Cu^{o}(s). In aqueous solution Cu(I) ion is rarely found and its compounds mostly insoluble or form stable complexes [66] as cu(I) is a soft ion, its complexes can be readily synthesized by using soft polarizable ligands such as thioethers, phosphines, nitriles, isonitriles, iodine ,cyanide and thiolates etc, form stable complexes with thiols and labile with amines. A broad range of coordination geometries is observed for these Cu (I) complexes. Most of the Cu(I) complexes are biologically important because they are able to reductively activate molecular oxygen (O_2) and as these complexes show a wide range of possible coordination geometries. As these complexes are labile means kinetically not stable due to this reason these show less radiopharmaceutical applications.

Due to the complete d orbital, Cu(I) complexes are diamagnetic and due to which they will be colorless. If a Cu(I) complex is colored, then the color may be the result of a charge transfer band or an internal transition in a ligand.

These Cu(I) tends to deposits in lipid rich tissues due to the Cu(I) binding tendency in hydrophobic environment which is due to the less thermodynamic stability in aqueous medium. Similarly the stereochemistry of Cu(I) is also unique, different from that of Cu(II) complex geometries, because of the d^{10} electronic configuration provides more symmetrical environment for Cu(I) and results in regular tetrahedral, trigonal and linear geometries [67,68,69].

Chemistry of Cu(II): While the most commonly occurred copper (II) oxidation state, in which the d orbital has 9 electrons present, which shows a very strong Jahn teller effects. Jahn- Teller distortion causes a splitting of e_g and t_{2g} orbitals, due to this reason Most of the Cu (II) complexes have square planar geometries. Usually the Cu (II) complexes show the electronic spectra which contain a single broad, poorly resolved band envelope/packet. This envelope is basically the indication of Cu (II) complexes in tetragonal complexes. These complexes are generally blue or green because of an absorption band in the 600-900 nm region of the spectrum.

1.12. Anti-cancer Activities of Various Cu(II) Complexes

Cancer is one of the major health concern problem and one of the major target for medicinal chemistry. Since DNA has defined as a target material for interaction of many small molecules. Although Platinum based complexes are initially used for treatment of several human cancers as chemotherapeutic agent, especially those of genitourinary origin [70,71,72], but due to their many side effects this interest has been shifted to non-platinum based complexes of different metal complexes as they show no or less side effects and same or better in cytotoxicity. Thus a large number of metals such as rhuthenium, gold, titinum, palladium, copper (II), nickel, germanium, cobalt are intensively being studied in place of platinum as chemotherapeutic agents. Further, detail studied provided that Cu (II) based complexes show a promising results as for cancer therapy and this idea is supported by a number of research articles which explain the synthesis and cytotoxic activities in detail for numerous Cu(II) based complexes [73,74].The coordination chemistry of copper (II) attracts much attention because of its biological relevance and its own interesting coordination chemistry such as geometry, flexible redox property, and oxidation state [75,76].

As there are so many transition elements found in different states but research study concentrated on copper metal in Cu(II) form due to the existence of two copper isotopes,64Cu and 67Cu , which are interestingly use in nuclear medicine. The 64 Cu isotope has ((half-life 12.8 h; β 655 keV; b 573 keV; g 511 keV and that of 67Cu has half-life 62 h; β 577, 484 and 395 keV; g 93 and 185 keV and 185 keV,) these two isotopes are largely used in diagnosis and as well in radioimunotherapy. In past time, most of the efforts have been done on these Cu (II) complexes with nitrogen donar ligands and their products have been passed through the process of clinical trials. As discussed before these Cu (II) complexes show kinetically inertness and this property is responsible for medicinal use. Therefore, copper (II) complexes with more complex ligands, can be used as model for other compounds which is largely used in nuclear medicine [60].

During the recent years, more interest was focused on designing the geometries and studying the properties of redox potential and spectroscopically active Cu(II)/I) and Ru(II/I) containing complexes as artificial chemical nucleases for DNA binding [49].

The chemistry of copper complexes have been deeply studied as they have a variety of applications/importance in different fields ranging from industrial catalysis to biomedical activities on the basis of their structure and reactivity relationship [77] a systematic study was done on multidentate ligands with S donar and phenyl moiety and their Cu(II) and Cu(I) complexes as their close resemblance to that of biological systems, that leads to spontaneous reduction of Cu(II) to Cu(I) while in absence of S donar atom, no spontaneous reduction will happen [9]. This is the reason that Cu (II) complexes that possessing macrocyclic structures, which presents their specific geometry and show their effective nuclease activities through interactions with DNA in different ways. Similarly, polynuclear Cu (II) complexes show their nuclease activities and their interaction modes have been studied deeply during recent years [48]. as copper is an essential element which is required for sustaining all biological systems in trace amount and the third most abundant element after iron and zinc [78], so very deeply studies were focused on the activities of Cu(II) and their important complexes with essential drugs inside the biological system [57], and in bioinorganic chemistry side, such type of interfering with the DNA and showing their activities as artificial nuclease, have left a survival interest in this field, such as a complexes of Cu(II) with aminoglycosides, which have the capability to cleave the DNA and RNA in both cases either H_2O_2,oxidizing agent present or absent. Another cytotoxic complex [Cu^{II}(pyrimol)(Cl)], which is in square planar form and behave as proficient agent in the absence of any reductant, for DNA cleavage. Similarly complexes of Cu (II) with linear or simple histidine ligands, demonstrate as effective nuclease for biological activities [48].

Transition metal Complexes containing 1,10-phenanthroline and related chelates have been extensively studied for different microbiological and pharmacological purposes and show a wide spectrum of anti-microbial activities and also results no toxicity to skin, subcutaneous tissues and mucous membranes [79,80].

Further, it has also been observed by studying the antitumor activities of Cu (II) complexes of different ligands that these activities were increased after incorporation of 1,10-phenanthroline ligand and also these ternary complexes show cytotoxic activities which were comparable to that of anti-cancer drug cis-platin.[81].

Similarly, the Copper that have ability to form complexes with 1,10-phenanthroline which are well known for their nuclease activities for DNA cleavage after studying their chemistry in the presence of nucleic acids. Basically these complexes

of phenantroline have the capability to inhibit viral activities by their interaction with DNA and thus prevent the formation of proviral DNA synthesis [49]. From these studies it has been observed that bis(1,10-phenanthroline) copper(II) complex superficially connects with DNA in two modes, as this complex consists of two planar rings, one ring is responsible for partial intercalation and the second ring responsible for weak interaction at minor groove of DNA. From more recent work of Thomas and coworkers on phenantroline analogue, it has been resulted that binding ability of bis-phen with DNA is more high as compared to mono phenanthroline complex [51].

Along with their benefits, these transition metal complexes containing phenanthroline and the bipyridine as ligands, have also exert some toxic effects on biological systems, as these are toxic to mice and rats [82], also show toxicity to rabbits and frogs [83].The toxic effects of these complexes also observed on enzymatic activities [84] and neuromuscular transmissions [85].

As Cu (II) complexes have been proved to be as an artificial nuclease which act as an efficient tool for the foot printing and sequence specific targeting of nucleic acid. Inspite of the intensively studied their interactions with DNA, but the binding mode of these complexes is still not known. Now a day, the researchers are trying to develop new ways of designing the geometries of these Cu complexes for more efficient and more useful catalysts for binding and cleaving the DNA, which are also known as artificial nucleases. For example the one of the well known complex of copper, CuU L-histidine is responsible for the cleaving the plasmid DNA at their physiological pH and specific temperature. The proteins which is a giant molecule, is composed of small structural units which we called as Amino acids. By studying the properties of these amino acids and their residues, it is well noted that capability of the Cu complexes to bind and show some reactions with the DNA, can be well judge from these studies. From these studies, it is revealed that these amino acids play an important role by controlling the active sites at and around of various metalloproteins by controlling the redox potential properties of metal ions, also help in the designing of geometries around a coordination metal center and also control the peptide chain through restriction their conformations. By studying the cyclic voltammetry data, it has been concluded that amino acids have the ability to promote the specificity for that molecules for their interactions at specific active sites through controlling and regulating the redox potential of that molecules [86].

There are few reports from where it has been deduced that Copper has the capability of forming a large number of complexes due to its variation in oxidation states in which a variety of copper (II) complexes have been apply as a synthetic chemical nuclease for the strand scission of duplex DNA hydrolytically and also for the analysis of DNA structure in solution [49,87]. Sigman and co-workers have reported that cationic complex $[Cu(phen)_2]^+$, that has been proved effective for the oxidative cleavage mechanism give a high priority to double stranded DNA the B-DNA effectively at minor groove in the presence of a reducing agent and molecular oxygen and by studying their antiviral activities ,it has also demonstrated that this complex inhibits proviral DNA synthesis very effectively [49].

The DNA binding studies of various Cu (II) complexes were carried out by using UV-Vis titrations, florescence, circular dichroic and viscosity measurements which clearly explain that some of the Cu (II) complexes interact with DNA preferably through groove binding, some cleave the DNA strands through hydrolytic pathway (validated by T4 ligase assay), interacts through major groove followed the oxidative mechanism, also bind to minor groove of DNA double helix [88,89,90].

The choice of coordinated ligands seem to be also very important along with the metal selection, as these coordinated organic ligands also an integral part of the biologically active complexes which designs the geometries for the complexes and exerts its own biological activities [73,74,91]. We decide the ligand for better chelation mostly O, S, N donar atoms as they mostly results in stable chelates for metals with different coordination modes, their affinity to form strong hydrogen bonds and their biological activities in human metabolism system. The inorganic, pharmaceutical and medicinal chemists are highly interested in developing of complexes with such type of active ligands, to design a good DNA binders [72,92,93].

1.13. DNA

DNA is abbreviated as “Deoxyribonucleic acid” that is composed of two long helical chains that are coiled around each other, made of polymers, the simple unit of which is known as nucleotides, the back bone is composed of alternating sugar and phosphate groups and these two strands are joined together through ester bonds. These two strands are anti-parallel to each other. These asymmetric ends of DNA strands are called the 5′ (five prime) and 3′ (three primegment). Basically DNA is a nucleic acid that

possesses the genetic informations in the form of instructions that are required for development and normal functioning of all living organisms except to RNA of viruses.

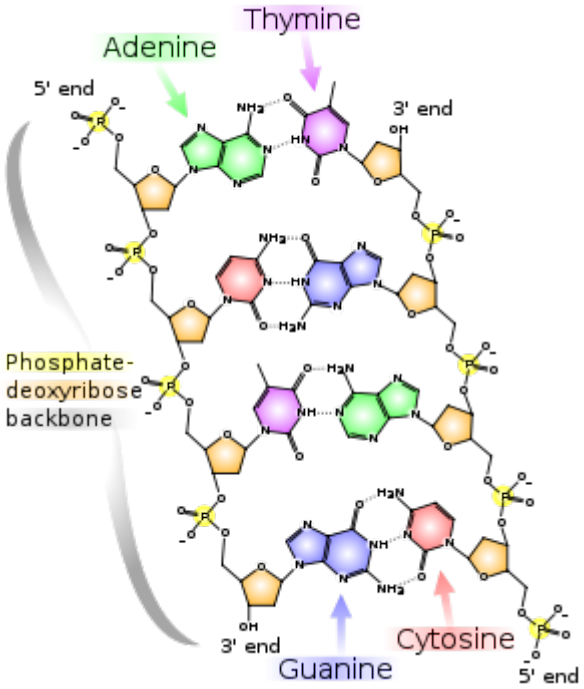


Fig 1.7: DNA double helical strand

DNA was for first time, discovered by James D. Watson and Francis Crick. These nucleotide molecules are very small, the DNA polymers may be made of a large number of these small repeating units, make them very large, e.g, the largest human chromosomes, is approximately 220 million base pairs long chain.

1.14. Grooves

The DNA double helixes are coiled around each other, resulting in space or grooves formation, between the strands. These grooves or spaces are next to the base pairs, which provide the binding site for small molecules. These grooves are of unequal sizes, because these strands are not directly opposite to each other, one is called major groove and other is minor groove. Size of the major groove is $22A^{o}$ and that of minor groove is $12A^{o}$.when the edges of base pairs are more near to each other in major groove, they will result in narrower the minor groove.

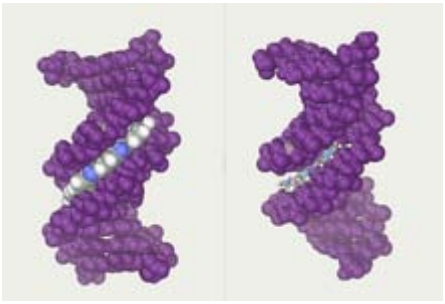


Fig. 1.8: Major and minor grooves of DNA.

Minor groove is a binding site for the dye. After performing a large number of biological experiments on DNA, this was confirmed that this DNA is basically key target inside the cell for interaction small molecules using as anticancer drug and DNA strands, because in cancer treatment these drugs damage the cancer cells, results in the inhibition of cell divisions and thus cause cell death. For understanding the mechanisms of the anticancer activities, studying interactions between the anticancer drugs and that of DNA is very important [94].

1.15. Techniques should be used

Various techniques have been used for explaining and studying the DNA binding interactions with small metal complexes. These techniques are electrochemical methods which include pulse polarography, cyclic voltammetry, square wave voltammetry, absorptive stripping voltammetry and potentiometric stripping spectroscopy etc., the molecular spectroscopy methods such as UV spectroscopy, fluorescence, circular dichroism etc., and various other techniques such as X-ray diffraction, dynamic viscosity measurements and high performance liquid chromatography.

Among these electrochemical, UV spectrophotometric and fluorescence methods have been largely used for studying such type of interactions because such type of interactions may be guided experimentally by studying the changes in intensity and or position of the peak responses.

The synchronous fluorescence spectroscopy (SFS) which was introduced by Lloyd who in 1971 introduced this in the field of forensic science, has got a great attention for this study, SFS was further modified with the addition of Vo-Dinh theory, in which the simultaneous scanning of excitation and emission outputs has been involved. In comparison to conventional fluorescence spectrum, this SFS has more sorts and give us more basic informations. In addition to this other advantages like narrowing

of the spectral band, simplification of spectra, and contraction of spectral range also obtained in this method. SFS require no physical separation and has been able to determine many components in a given mixture.

The basic purpose of the present work was to develop different analytical techniques such as cyclic and differential pulse voltammetry as well as UV–visible and fluorescence spectroscopies in combination with chemometrics methods for studying the purpose of neutral red dye (NR), which has been used as a clue for binding studying between the bis(1,10-phenanthroline)copper(II) complex ($[Cu(phen)_2]^{2+}$) and DNA [51].

As discussed before in detail, the Cu (II) complexes have the capability to show a variety of interactions with DNA and antioxidant activities DPPH, such type of interactions are intensively studied by using various techniques such as UV-visible spectroscopy, cyclic voltammetry, viscometry, laser light scattering in my research work.

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Chapter − 2

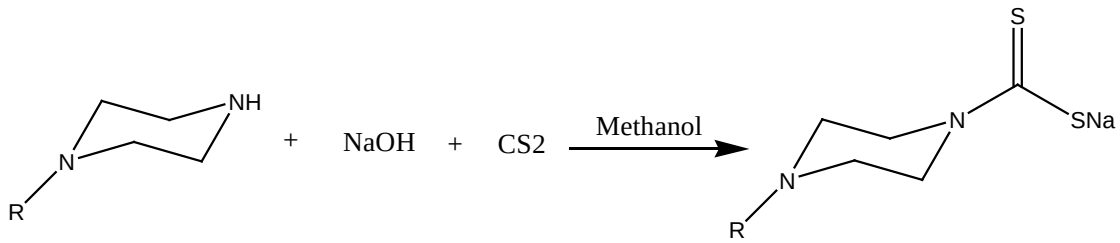
Experimental

Chapter – 2

EXPERIMENTAL

2.1. General Procedure for Preparation of Piperazine-1-carbodithioate derivatives: (Na-L)

To the solution of substituted piperazine (1.2 g, 5.78 mmol) in methanol, an equimolar of sodium hydroxide (0.23 g, 5.75 mmol) dissolved in distilled water (5 mL) was added and stirred for 1 hour. After this, CS_2 (0.35 mL, 5.78 mmol) in methanol was added dropwise through a dropping funnel. The whole system was ice covered and stirred for 8 hours at room temperature (25±1^oC). The precipitate obtained, was washed with diethyl ether and dried under IR lamp (scheme 1).



R = NO_2—C_6H_5

Scheme 2.1: Synthesis of piperazine-1-carbodithioate derivatives

Weight of filtrate obtained was 1.697 g.

m.p of filtrate = 288—290^oC

2.2. Synthesis of Complex-1

$CuCl_2.2H_2O$ (0.119 g, 0.698 mmol), was dissolved in ethanol (25 mL) and then bipyridyl (0.109g, 0.760 mmol) was added to warm ethanolic solution, result in color changes from light green to yellowish green, and the mixture was heated with stirring for 30 minutes. An ethanolic solution of sodium salt of ligand (0.3 g, 0.612 mmol) was then added dropwise, color change to dark brown. The mixture was heated under reflux with stirring for 8 hours (scheme 2). On cooling the solution was precipitated and was filtered off by using wattman filter paper. After filtration, dark brown precipitate and that of dark green color filtrate was obtained. The filtrate was left for slow evaporation at room temperature. %Yield (58.24). Soluble in most of the organic solvents.

2.3. Synthesis of Complex-2

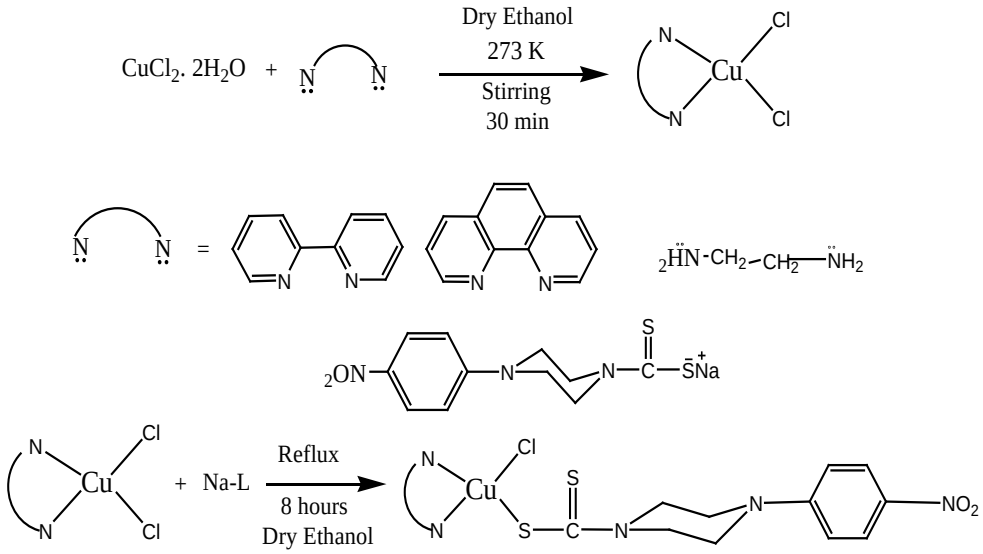
Complex **2** was synthesized using the same procedure adopted for complex **1.** $CuCl_2.2H_2O$ (0.12g, 0.704 mmol) 1,10-phenanthroline monohydrate (0.14g, 0.704 mmol) and 4-NPPZD ligand (0.345g, 0.704 mmol). The precipitate was decomposed at 265.5^oC. The precipitate was re-dissolved in ethanol and *n*-hexane mixture and was kept for crystallization at room temperature. Soluble in most organic solvents.

2.4. Synthesis of Complex-3

Complex **3** was synthesized using the same procedure adopted for complex **1.** $CuCl_2.2H_2O$ (0.335g, 1.96 mmol) ligand-salt (0.6g, 1.96 mmol) and ethylenediammine (0.131mL, 2.0 mmol)

The melting point of precipitate was noted as 275-279^oC.

2.5. General Procedure for Complex Synthesis



Scheme 2.2: Synthesis of N and S donor mixed Ligands complexes of Cu (II)

2.6. DNA Interaction Study by Using UV-Visible Spectroscopy

CT-DNA was dissolved and stirred for overnight in double deionized water (pH = 7.0) and kept at 4^0C for less than 4 days. The nucleotide to protein (N/P) ratio, 1.8-1.9, was obtained by measuring the absorbance at 260 nm and 280 nm (A_{260}/A_{280}=1.9). which showed that DNA was free from protein. The DNA concentration was measured by using molar coefficient of 6,600 $M^{-1}cm^{-1}$ (260 nm) for CT-DNA, and was found to be 134.5 µM. The compound was dissolved in freshly dried ethanol having concentration 5 mM solution (50 mL).

The UV absorption titration was performed keeping the concentration of compound constant while concentration of CT-DNA was varyingstepwise. Equivalent concentration of CT-DNA was added to the sample solution and to that of reference solutions, for the purposes to eliminate the absorbance caused by CT-DNA itself. The compound DNA solution was incubated or kept for a while before measurements were recorded on spectrophotometer. Then the absorption spectra were recorded by using cuvettes of 1 cm at room temperature (298 K). This processes repeated after some time,but same results were obtained, which confirms the drug-DNA interaction. Based upon the variation in absorbance, the intrinsic binding constant (K_b) of our sample with DNA can be calculated by using **Benesi-Hildebrand Equation 2.1.,**

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} \cdot \frac{1}{K_b[DNA]} \qquad \textbf{Eq. 2.1}$$

Where K_b is the binding/association constant, A_o and A are the absorbances of pure drug and drug-DNA solution respectively, ε_G and $\varepsilon_{H\text{-}G}$ are the molar absorption coefficients of drug and drug-DNA complex respectively. The intrinsic constant (K_b) were obtained from intercept to slope ratios of Ao/A-Ao Vs 1/ [DNA] plots. While the Gibbs' free energy (G) were caculated by using the following equation 2.2,

$$\Delta G = -RT \ln(K) \qquad \textbf{Eq. 2.2}$$

Where R gas constant (8.3141 $JMol^{-1}K^{-1}$) and T is the absolute temperatur (298K).

2.7. Scavenging Effect on 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

Habila et al., procedure for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was adopted **(2010)** [1] with some modification to study the antioxidant activity.. The solution of 2,2-diphenyl-1-picrylhydrazyl was obtained by dissolving it in 100 mL ethanol. A solution of our sample required for activity (100 µg) was the prepared in ethanol. Different concentrations solutions of compounds were prepared and then add 1 mL DPPH to each solution tubes and reference tubes, so as to nullify the absorbance caused by DPPH itself. The variation in absorption was noted at 517 nm as DPPH absorb light at this wavelength and gave intense purple color. Then calculated the % Inhibition, from which IC_{50}/EC_{50} valve was calculated for each sample.

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Chapter – 3

RESULTS AND DISCUSSION

Chapter – 3

RESULTS AND DISCUSSION

3.1. Physical Data of the Ligand and its Copper (II) Derivatives

The physical data for all the complexes synthesized was given in the Table 3.1. The complexes synthesized were seemed to be pure and were mostly soluble in DMSO, Ethanol and methanol. The melting points were given and mostly they decomposed**.**

Table 3.1: Physical Data of the Ligand and Cu (II) complexes

Comp. No.	Molecular Formula	Molecular Mass	Physical State	Melting Point (^oC)	Solubility
Na-L	$NaO_2S_2N_3C_{11}H_{12}$	204.98	Yellow powder	288-290	Turbid in acetone
1	$CuO_2ClS_2N_5C_{21}H_{20}$	535.16	Bluish powder	265.5 decomposed	DMSO, ethanol
2	$CuO_2ClS_2N_5C_{23}H_{20}$	561.57	Bluish powder	260-282	Ethanol, acetone, DMSO
3	$CuO_2ClS_2N_5C_{13}H_{20}$	441	Blue Violate	275-279	Methanol

3.2 Elemental Analysis

The elemental analysis of complexes was found in good agreement to the calculated data (Table 3.2), and thus indicated the formation of complexes.

Table 3.2: Elemental Analysis of Cu (II) complexes

S. No.	Empirical formula	%C Cal. (found)	%H Cal. (found)	%N Cal. (found)	%S Cal. (found)
Na-L	$C_{11}H_{12}N_3NaO_2S_2$	43.27 (43.25)	3.96 (3.94)	13.76 (13.75)	21.00 (20.99)
1	$CuO_2ClS_2N_5C_{21}H_{20}$	49.47 (49.45)	5.02 (5.01)	12.02 (12.0)	10.73 (10.70)
2	$CuO_2ClS_2N_5C_{23}H_{20}$	50.93 (50.91)	4.10 (4.90)	11.88 (11.86)	10.88 (10.87)
3	$CuO_2ClS_2N_5C_{12}H_{20}$	33.11 (33.10)	3.85 (3.83)	14.85 14.83)	20.40 (20.39)

3.3. The FT-IR Spectroscopy of Ligand-salt and Complexes

FT-IR provides valuable information about the formation of complexes and the data collected in the Table 3.3, indicate complexes formation. Among them the C-N and C-S stretching vibrations are of particular interest as these peaks indicate the mono and bidentate binding modes of 1,1-dithioate moiety. The single peak observed at 900-1000 cm^{-1} is due to the C-S stretching vibration, usually indicates the bidentate mode of dithioate moiety whereas the presence of two bands with the separation of greater than 20 cm^{-1} represent the mondentate binding mode of the bonded ligand. In our present study the presence of two bands at 1106 cm^{-1} and 1036 cm^{-1} are the asymmetric and symmetric stretching vibrations respectively for C-S bond indicates that here the 1,1-dithioate moiety act as a monodentate ligand to Cu [1,2,3,]. The band at 1426 cm^{-1} is due to the C-N stretching vibration, which indicates the partial double bond character for C-N [3]. The band at 1585 cm^{-1} was due to the ν(C=N) present of 1,10-phenanthroline which was shifted to lower frequency upon complexation with Cu atom [4,5]. The Cu-N vibration was observed in complexes at 530-560 cm^{-1} [6,7]. The Cu-S stretching vibration was appeared in the range of 380-450 cm^{-1} which indicated the complex formation [8]. In these complexes a band in IR region at 330-355 cm^{-1} was assigned to the Cu-Cl stretching vibration [5].

Table 3.3: FT-IR (cm^{-1}) data for Cu (II) complexes

Compound	ν(CSS)	ν(N-CS_2)	ν(Cu-S)	ν(Cu-N)	ν(C=N)
1	1158 (asym) 1012(symm)	1438	426	555	1583
2	1106(asym) 1036(symm)	1456	426	540	1585
3	1047(asym) 1020(symm)	1472	420	550	-

3.4. UV-Visible Spectroscopy

3.4.1. UV-Visible Spectroscopy of Complex-1

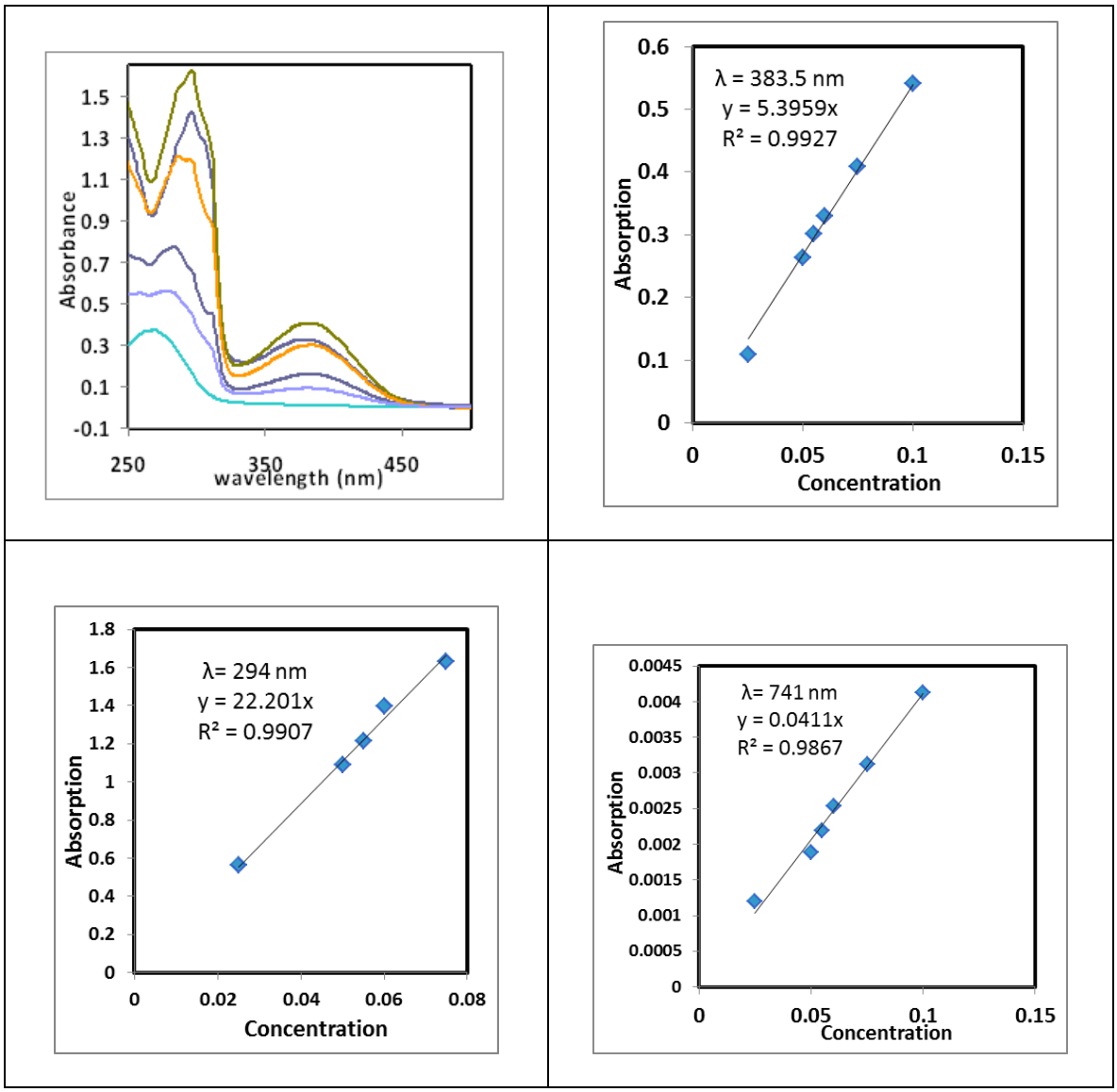


Fig. 3.1: UV-Visible Spectrum of the complex-1.

In the Fig: 3.1 for complex-**1**, the spectra shows n-π* and π-π* transitions at 3403 cm^{-1} and 34965 cm^{-1} respectively and ε calculated for these bands from t he slopes, are 21709 $M^{-1}cm^{-1}$ and 22000 $M^{-1}cm^{-1}$ respectively. This n-π* indicates the intra ligand transition which shows red shift from 286 nm to 294 nm upon complexation with copper [9]. These two π-π* are due to the presence of aromatic rings in the complex corresponds to the intra-ligand transitions. The band at 26109 cm^{-1} basically explains the charge transfer phenomenon, which corresponds to the transfer of electrons from sulfur atom to Cu metal and the valve calculated for this band from the slope i s 5509 $M^{-1}cm^{-1}$, which lies in expected range [9,10]. The characteristic d-d transition observed

for the complex, is in the range of 740-935 nm (use identical unit). This is weak and broad and corresponds to $^2B1g \rightarrow {}^2Eg$ in the square planar complexes; the value calculated for this is 54.5 $M^{-1}cm^{-1}$ [9,10]. As no band was observed below the 10,000 cm^{-1} excludes the possibility for tetrahedral geometry [10]. Since only one d-d transition band observed in both the complexes-1 and 2, it means that in both complexes, central metal atom is in the same environment [11].

Table 3.4: Possible transitions in complex-1.

Transitions	λ (nm)	ν (cm^{-1})	Є (M^{-1} cm^{-1})
d-d	741	13495	54.5
CT	383	26109	5509
n – π*	294	34013	21709
π – π*	286	34965	22000

3.4.2. UV-Visible Spectroscopy of Complex-2

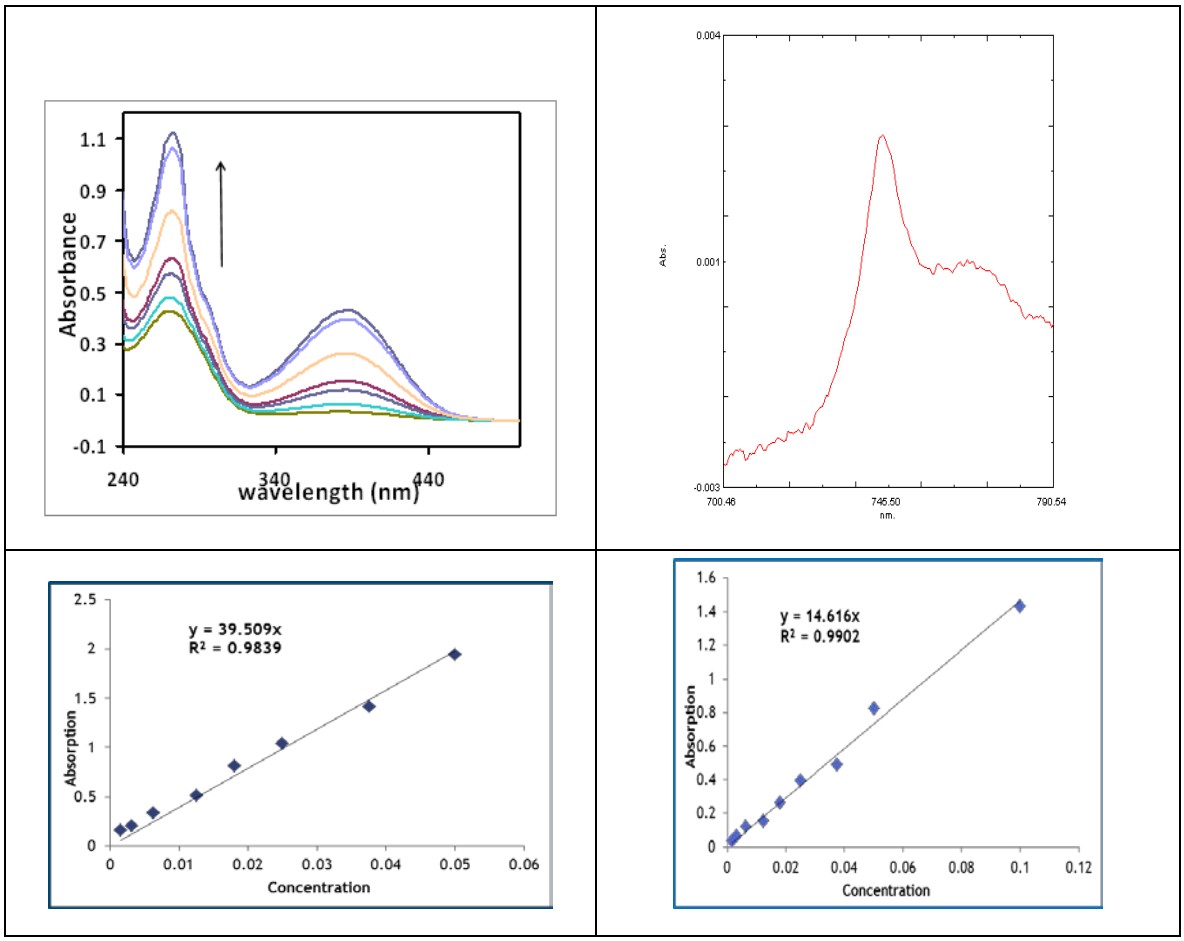


Fig. 3.2: UV-Visible spectrum of the complex-2.

Complex-**2** showed π-π* transitions at 36764 cm^{-1} and 43956 cm^{-1} and the ε value calculated for these from the slope, are 42440 $M^{-1}cm^{-1}$ and 50920 $M^{-1}cm^{-1}$, respectively. The band at 26041 cm^{-1} is basically the charge transfer phenomenon from S atom to the central metal atom Cu, and the ε calculated for this band from the slope is 15720 M^{-1} cm^{-1}. The characteristic weak broad band observed in the visible region of the spectrum at 760-935 nm (13440 M^{-1} cm^{-1}) is due to the d-d transition for copper complex corresponds to $^2B1g \rightarrow {}^2Eg$, which suggests the square planar geometry for the complex [9,10], and as there is no band observed below 10,000 cm^{-1}, which omit the possibility of the tetrahedral geometry for the given complex [10].

Table 3.5: Possible transitions in Complex 2.

Transitions	λ (nm)	ν (cm^{-1})	Є ($M^{-1}cm^{-1}$)
d-d	746 nm	13441	120
CT	384	26042	15720
π – π*	272	36765	42440
π – π*	227.5	43956	50920

3.5. CT-DNA Binding Study of Complex-1 and 2

The drug-DNA interactions can be carried out by using UV-Visible absorption spectroscopy by monitoring the changes in absorption properties of drug or the DNA molecules. The drug-DNA interaction can be studied by comparision of UV-Visible spectra of free drug and drug-DNA complexes, which must be different from each other if there is any interaction. Drug binds with DNA through intercalation, usually results in hypochromism and bathochromism. Because of the intercalative mode involve a stacking interaction between an aromatic chromophore and base pairs of the DNA, the extent of hypochromism usually parallel the intercalative binding strength.

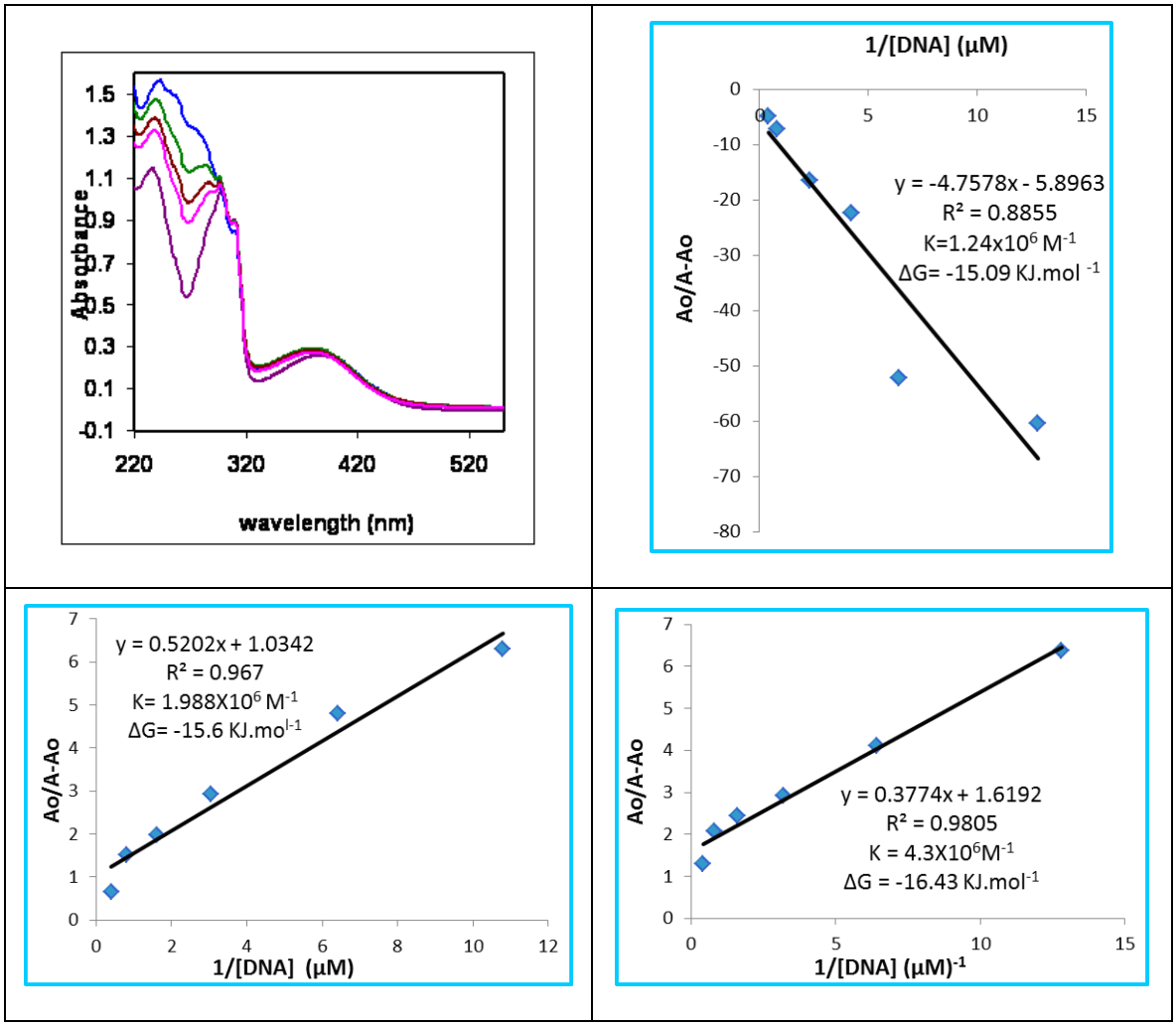


Fig. 3.3: UV-Visible absorption spectra for 0.005mM complex-1 (50:50 in water and ethanol) in absence (a) and presence of 0.078 mM (b), 0.156 mM (c), 0.3125 mM (d), 0.625 mM (e), 1.25 mM (f) and 2.5 mM (g) CT-DNA. The arrow direction shows the increasing concentration of CT-DNA.

The absorption spectra of complex-**1** in absence and presence of CT-DNA is shown in Fig.3.3, there exists three bands in the spectrum. The addition of CT-DNA a hyperchromism at 286 nm and 383.5 nm, while hypochromism effect at 294 nm observed. These spectral characteristics suggest that the sample binds with CT-DNA either intercalative mode or groove binding mode. After intercalating the base pairs of DNA, the π orbital of the intercalated ligand may couple with π orbital of the base pairs, thus decreasing the π-π* transition energy and further results in the slight bathochromic shift. The coupling of a π orbital with partially filled electrons decreases the transition probabilities of electrons, hence result in hypochromism. Another phenomena that is observed at 286 nm and 383.5 nm, is hyperchromism,, which is due to the groove

binding mode of the sample with the DNA helix. This is because of unwinding of the double helical strands of DNA which consequently increased the absorbance at those specific areas. It may be further concluded that sample may interact at minor grooves, typically at N3 of adenine and O2 of thymine, through H-bonding with bases. Based upon the variation observed in the absorbance, the intrinsic binding constant (K_b) and Gibbs' free energy can be calculated as by using following equations (3.1) and (3.1) respectively,

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} \cdot \frac{1}{K_b[DNA]} \qquad \textbf{Eq: 3.1}$$

And $\Delta G = -RT \ln (K)$ **Eq: 3.2**

The binding constant (K_b) calculated at those specific wavelengths, are 1.24 X 10^6 M^{-1}, 1.988 X 10^6 M^{-1}, and 4.3 X 10^6 M^{-1} respectively. Similarly, the Gibbs' free energy ΔG at those wavelengths are -15.9kJ.mol^{-1}, -15.6 kJ.mol^{-1} and -16.43 KJ.mol^{-1} respectively. Interestingly, binding constant (K_b) obtained for Cu (II) complexes is higher than ordinary Cu (II) complexes containing 2,2-bypridine (K_b= 3.24 X 10^4 $M^{-1}cm^{-1}$ for $Cu(bipy)_2^{+2}$) [12,14]. From the negative value of Gibbs' free energy, it has been decided that the interaction of drug with CT-DNA is a spontaneous process.

The absorption spectrum of complex-**2**, in absence and in the presence of CT-DNA is shown in the Fig: 3.4 there exits three bands at 272 nm, 386 nm and 744 nm for free complex-**2**. With the addition of CT-DNA, there is a change occur in the spectra of complex-**2**, which means that there is some sort of interaction between sample and CT-DNA. Based on the changes observed, an intercalative mode and groove binding can be assigned. The absorption increases with the increasing concentrations of DNA which shows the hyperchromism effect which is either due to the denatured/fragments or due to the unwinding of the double helix. The two strands in DNA mainly bind each other through H-bonds and hydrophobic effects between the complementary bases. These interaction forces restrict the resonance for aromatic rings, but when these forces are broken, the two strands got separated and results in randomly coiled conformations. The free nitrogenous bases absorb more UV light and results in the hyperchromism in the spectrum. There is a possibility that complex-**2**, may bind with DNA double helix through external groove and then result in unwinding of the double helical structure for DNA which result in hyperchromism [15].

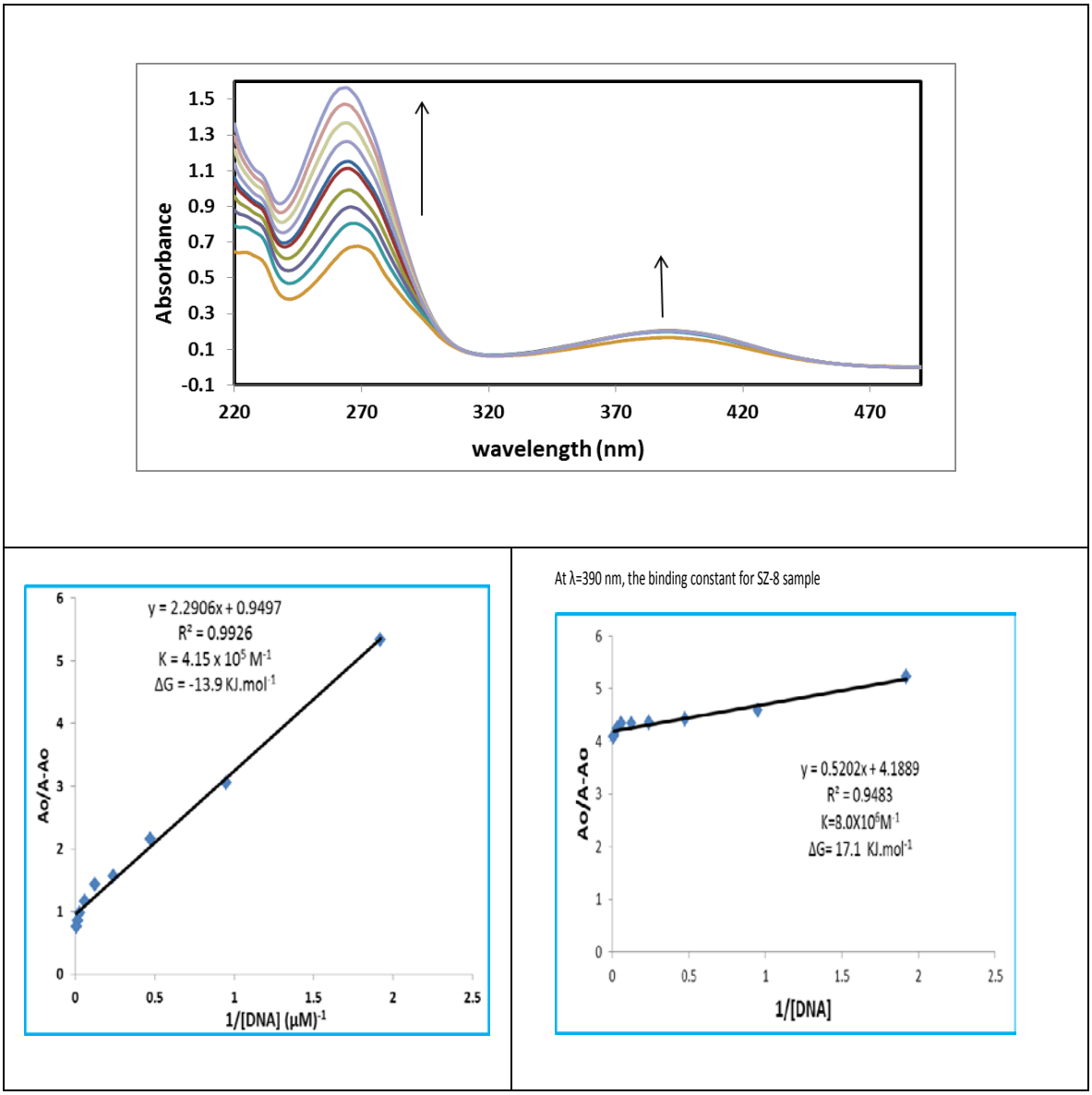


Fig. 3.4: Absorption spectra of 325 nM Complex-2, in the absence (a) and in the presence of 0.52 µM (b), 1.051 µM (c), 2.1 µM (d), 4.2 µM (e), 8.41 µM (f), 16.82 µM (g) CT-DNA. The arrow direction shows the increasing concentration of CT-DNA.

The binding constants (K_b) was found 4.15 X 10^5 M $^{-1}$ and 8.0 X 10^6 M^{-1} for λ_1 and λ_2 respectively and similarly the Gibbs' free energy -13.9 kJ.mol^{-1} and -17.1 kJ.mol^{-1} for λ_1 and λ_2 respectively. So interaction of drug with CT-DNA, is a spontaneous process as evident from the negative value of Gibb's free energy.

3.6. Assays for DPPH Free Radical Scavenging Activity

Experimental procedure for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was carried out according to the procedure described by Habila J. D et al., (2010) [13]. Different concentrations of complexes 1 and 2, and standards (Ascarbic acid, Gallic acid) were prepared in ethanol. 167 µg/mL solution of DPPH was prepared in ethanol, 1 mL of DPPH was added to different solutions i.e, 100, 50, 25, 12.5, 6.25, and 3.125 (µg/mL) of standard solution (ascorbic acid) and test sample, and then solutions was incubated at 37°C for 30 minutes and the absorbance at 517 nm was measured (Pharmaspec UV-1800 UV-Visible Spectrophotometer Schimadzu). % anti-oxidant activity was calculated by using the Equation 3.3

$$\%\text{Inhibition} = (A_0 - A/A_0) \times 100 \qquad \text{Eq: 3.3}$$

Then IC_{50}/EC_{50} value was calculated by the help of EZ fit® enzyme kinetic software.

3.6.1. Anti-Oxidant Activity study for Ascorbic Acid

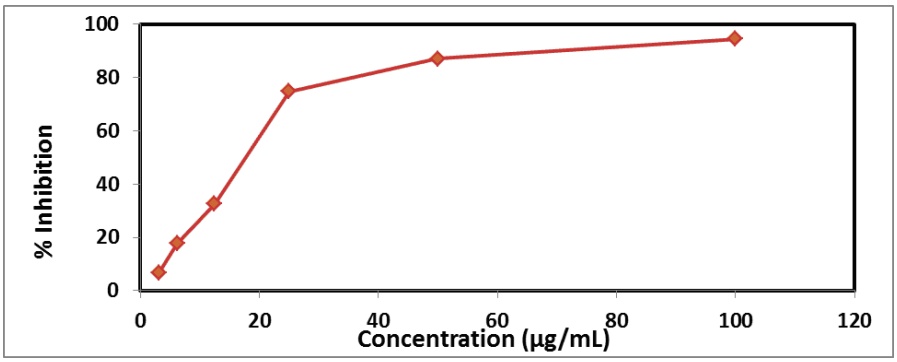


Fig. 3.5: Anti-oxidant activity of Ascorbic acid

Table 3.6: Anti-Oxidant Activity for Ascorbic acid

Conc. (µg/mL)	Absorbance	% Inhibition	IC_{50} / EC_{50}
Pure DPPH	0.661	–	17.76
3.125	0.639	6.57	
6.25	0.609	17.69	
12.5	0.299	32.57	
25	0.099	74.67	
50	0.052	86.89	
100	0.048	94.34	

3.6.2. Anti-Oxidant Activity Study for Complex-1

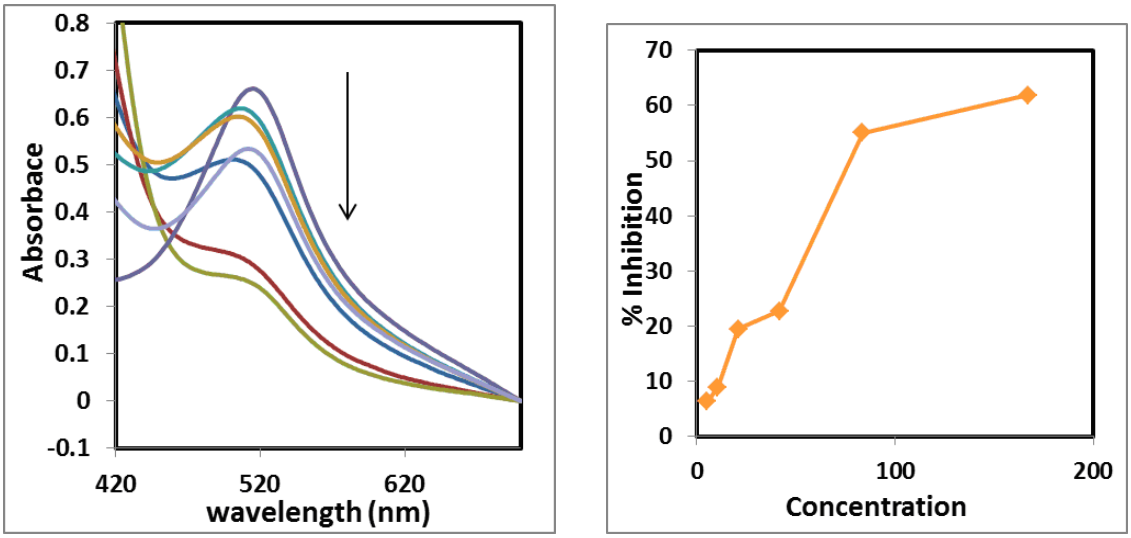


Fig. 3.6: Antioxidant activity of complex-1

Table 3.7: Anti-Oxidant activity and IC_{50} for complex-1.

Conc. (µg/mL)	Absorbance	% Inhibition	IC_{50} / EC_{50}
Pure DPPH	0.661	–	29.54
3.125	0.619	6.35	
6.25	0.602	8.9	
12.5	0.532	19.52	
25	0.511	22.7	
50	0.297	55.06	
100	0.252	61.87	

3.6.3. Anti-Oxidant Activity Study for Complex-2

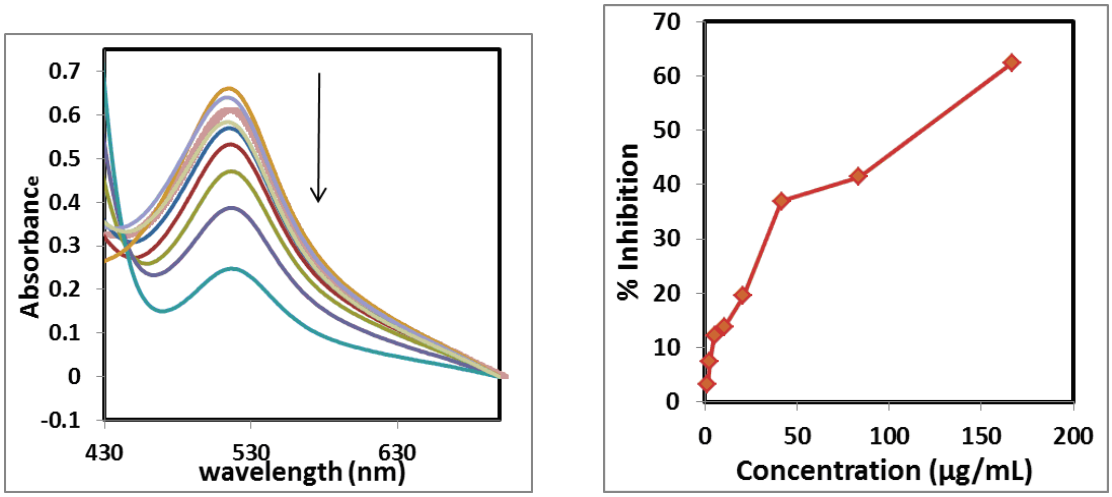


Fig. 3.7: Anti-oxidant activity of complex-2

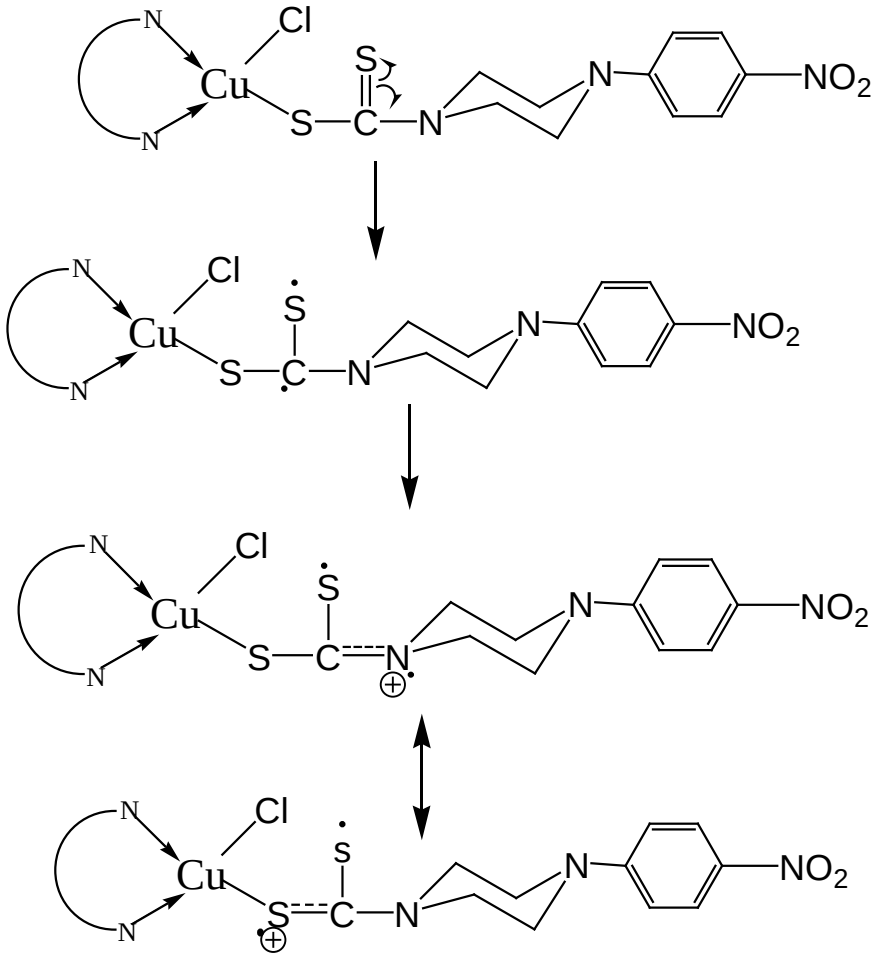
Table 3.8: Anti-oxidant activity and IC_{50} for complex-2.

Conc. (µg/mL)	Absorbance	% Inhibition	IC_{50} / EC_{50}
Pure DPPH	0.661	-	37.874
3.125	0.580	12.25	
6.25	0.573	13.76	
12.5	0.532	19.51	
25	0.417	36.91	
50	0.387	41.45	
100	0.248	62.48	

Table 3.6 shows the anti-oxidant activities of ascorbic acid and fig. 3.6 and 3.7 show anti-oxidant activities for (complex-**1** and **2**) respectively in ethanolic solutions. Graphs of the ascorbic acid and samples were plotted between concentrations and % Inhibition. The latter caused by our samples have very low values. Complex-**1** has low IC_{50} value than complex-**2**, means that complex-**1** show high anti-oxidant activity.

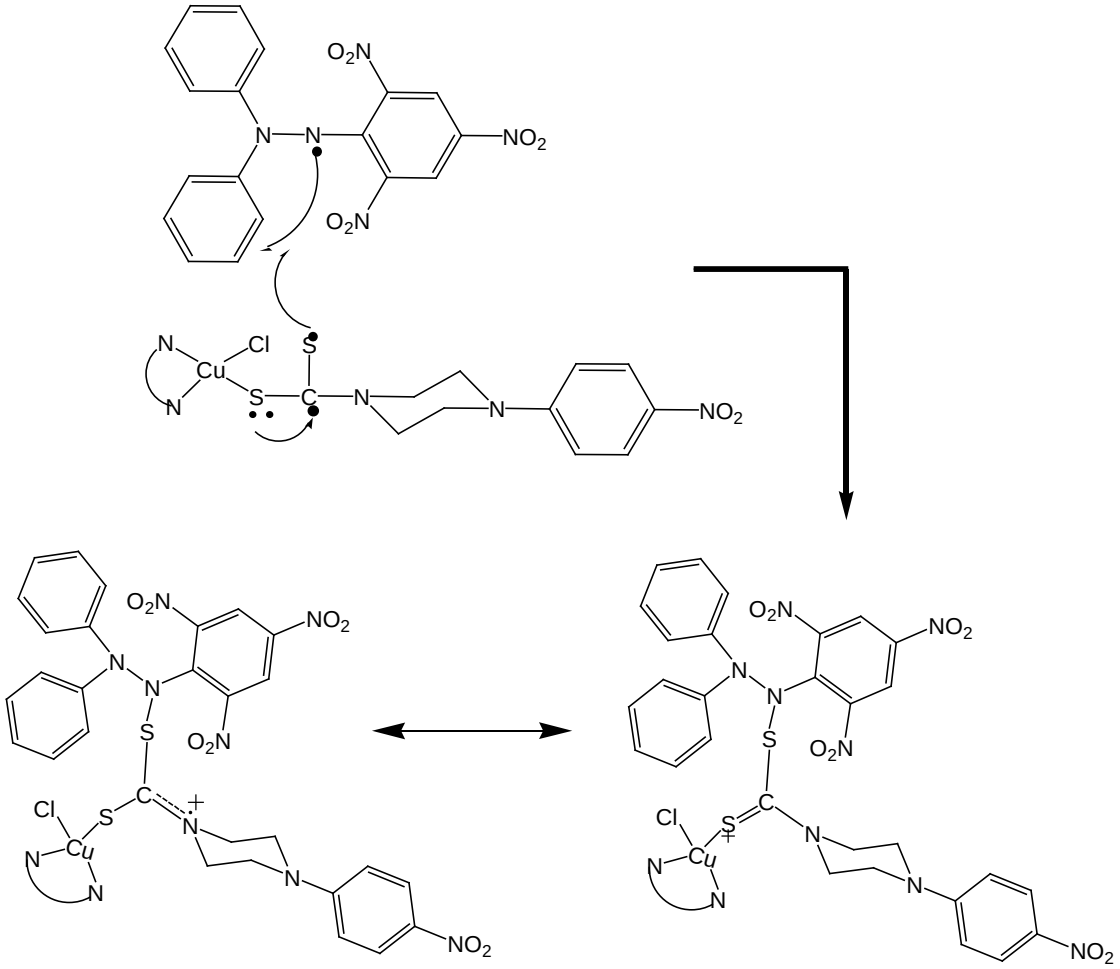
3.6.4. Proposed Mechanism for Anti-Oxidant Activity

According to Capto-Dative effect, the center at which the free radical should formed, must be connected to heteroatoms which have the ability to stabilize the free radicals formed either by donation of electrons or withdrawing the electrons. In synthesized complexes as C=S is connected to a N atom on one side and a S atom on other side, have high ability to form free radical at C=S center. A free radical formed at CS center is stable because of the two resonance forms formed (Scheme 3.1). There is a hemolytic cleavage between C=S in synthesized complexes, and the resultant free radical can be stabilized by either donating electrons by a N atom or a S atom, result in charge free radicals.



Scheme 3.1: Canonical forms of resultant synthesized complexes

These free radicals are able to interact with DPPH free radicals and have the ability to scavenge these DPPH free radicals as shown in scheme 3.2, and the resultant free radical is stabilized through different canonical forms.



Scheme 3.2: Proposed mechanism for anti-oxidant activities

3.7. Cyclic Voltammetry and Redox Mechanism

The cyclic voltammetry is the potentiodynamic electrochemical technique that is used for the study of qualitative information about electrochemical reactions, also provides information about thermodynamics and kinetics of heterogeneous electron transfer reactions and electron transfer process on electrode [16,17,18].

Cyclic Voltammograms of the complex-1 in ethanol shows well defined redox properties as shown in fig. 3.7 below, corresponding to the formation of different intermediates. The potential range copper (II) complexes was studied in between +1.5 and -2.0V. Before conducting and plotting the graphs, nitrogen gas was passed for 5

minutes [20], to remove oxygen wave in voltammogram. The cyclic voltammograms were obtained in different mediums at a scan rate of 100 (mV/S) and their mechanisms are described in detail as shown in below schemes.

At **pH= 3**, the potential window was kept in the range of -0.6 to +0.3 volt. Starting in positive direction, two prominent anodic peaks were observed. One oxidizing peak at -0.09 volt and second at 0.16 volt (scheme. 3.4), while during backward scanning, four reduction peaks are observed. One prominent peak at 0.01V, second at -0.15V, third peak at -0.28V and fourth peak at -0.5V (schem. 3.3). In oxidation, two electrons and two protons are involved while during reductions, one electron and one proton is involved. The detail mechanisms showed below in scheme 3.3 and 3.4.

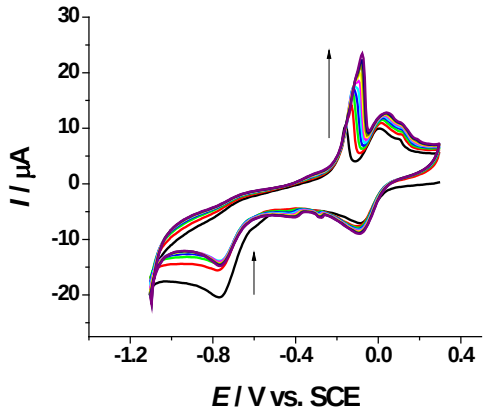


Fig.3.8.1: CVs (scan 1-10) at 100 mVs-1 at pH 7

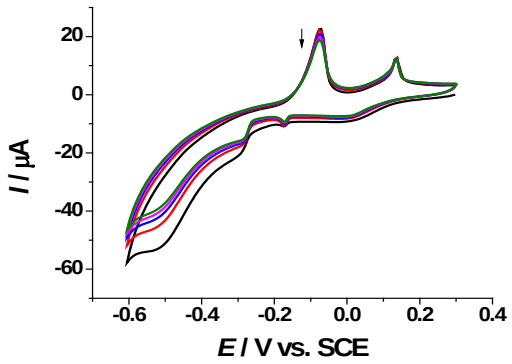


Fig. 3.8.2: CVs (scan 1-5) at 100 mVs-1 at pH 3

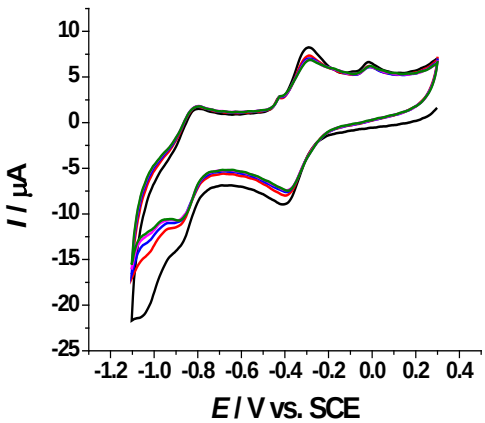


Fig. 3.8.3: CVs (scan 1-5) at 100 mVs-1 at pH 10

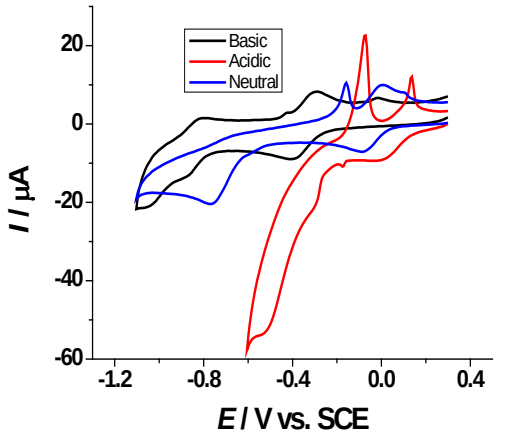
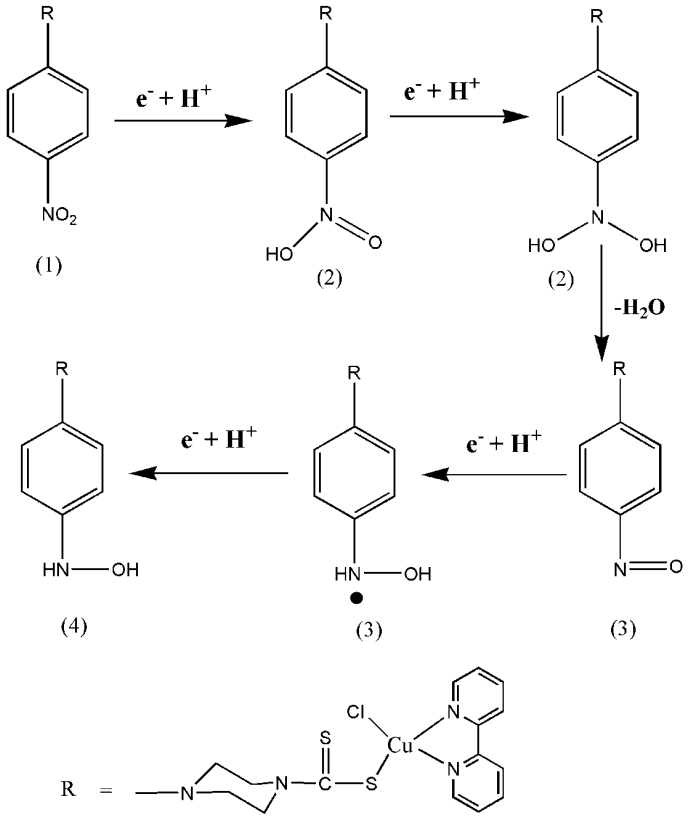
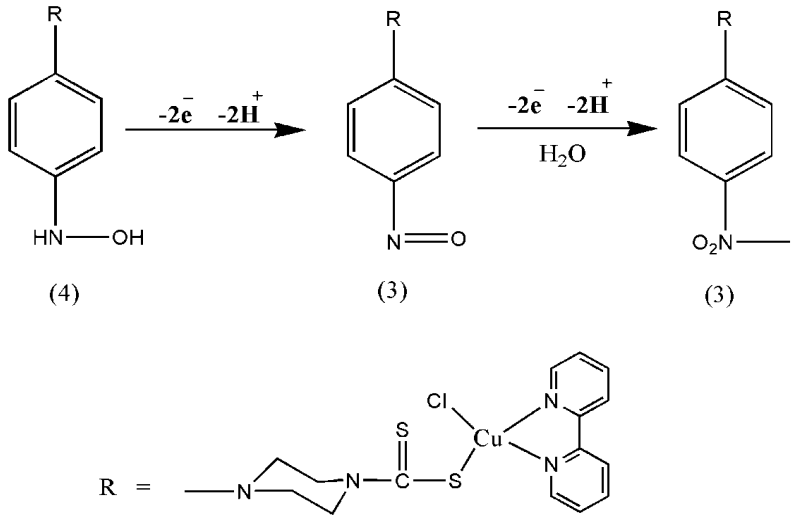


Fig. 3.8.4: combined

Fig. 3.8: Cyclic Voltammograms for complex-1



Scheme 3.3: Reduction in acidic medium

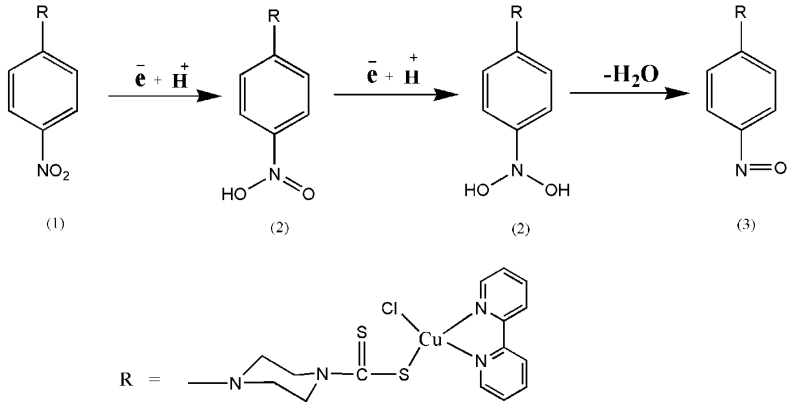


Scheme 3.4: Oxidation in acidic medium

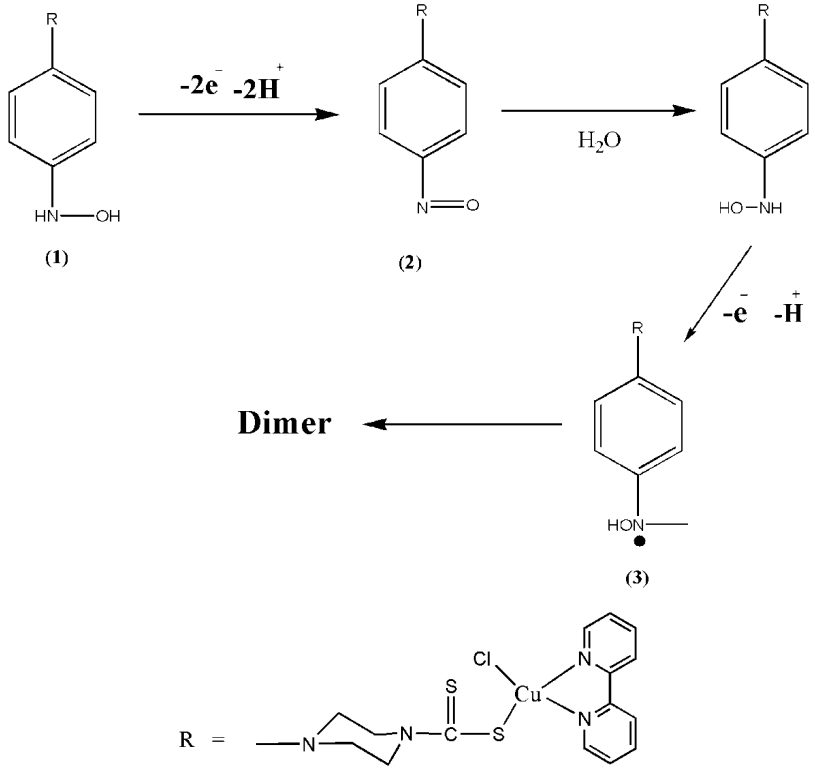
At **pH = 7,** during forward scan, the first oxidizing peak shifted from-0.09V to -0.17V and the second peak is shifted to +0.08 volt, which shows that the neutral medium has further enhance the oxidation of the complex-1 or in another words at neutral medium the oxidation is more easier as compared to acidic medium. While in back scan, two reduction peaks are observed at -0.05 and -0.76 volt, the shift in these two

peaks towards more negative potential shows that thee reduction is difficult in neutral medium as compared to acidic medium. The scan rate was maintained at 100 mV/S. The scheme 3.5 and 3.6 show the detail mechanisms for complex-1.

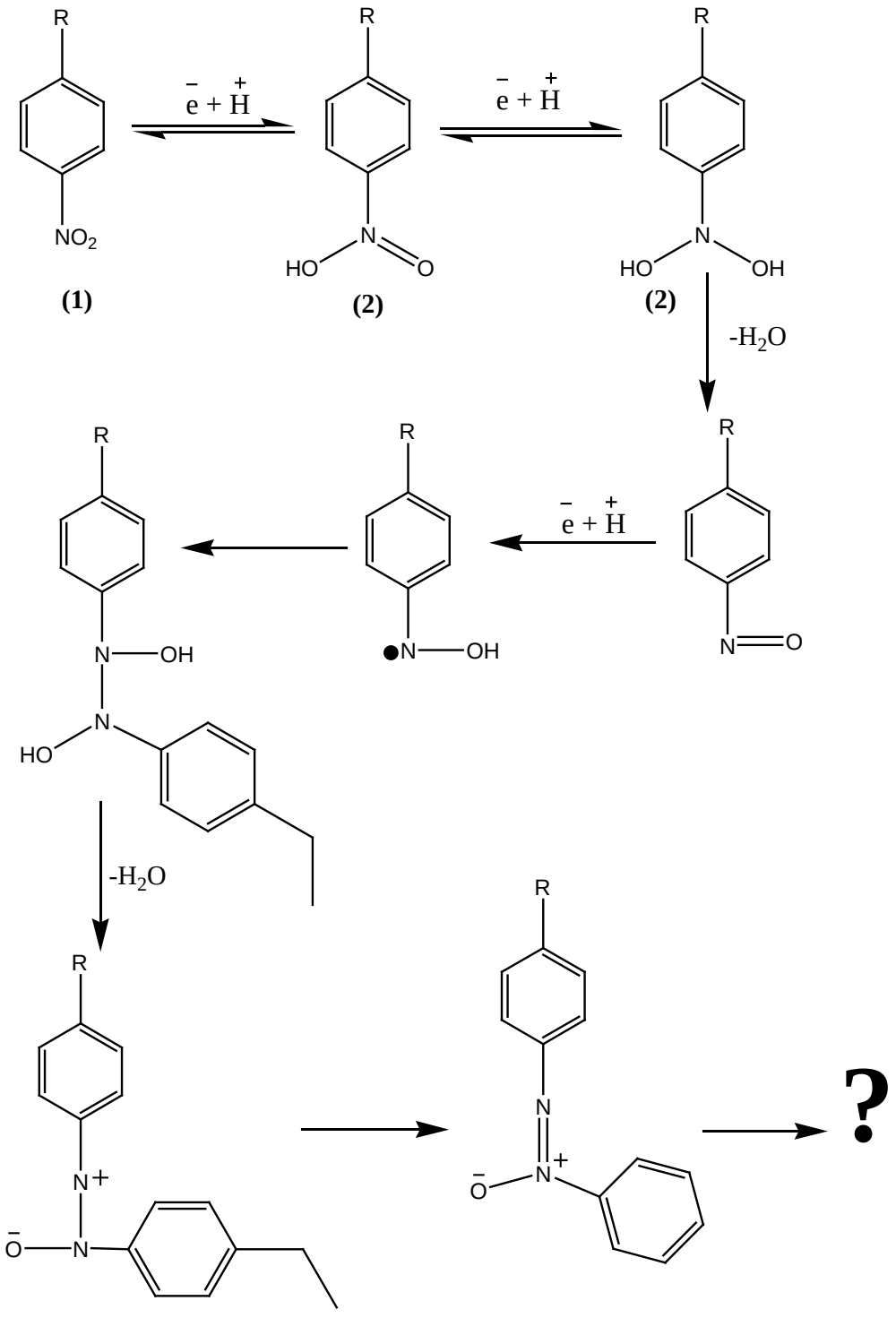
At **pH=10**, during forward scan, three oxidizing peaks are observed at +0.01 V, -0.3V and -0.8 volt, while in back scan, two prominent reduction peaks are observed at -0..35V and -0.83 volt, and also a small dent was observed -1.05 volt, but the detail mechanism is still not clearly observed. The scheme 3.7 shows the detail mechanism in basic medium.



Scheme 3.5: Reduction in neutral medium



Scheme 3.6: Oxidation in neutral medium



Basic Medium

Scheme 3.7: Mechanism in Basic medium

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CONCLUSION

- A series of Cu(II) 4-(4-nitrophenyl)piperazine-1-carbodithioate complexes have been synthesized and characterized by using UV-Visible Spectroscopy, FT-IR, elemental analysis and Cyclic Voltammetric techniques.
- From FT-IR data, the characteristic peaks observed at 1583 and 1585 cm^{-1} for C=N in 2,2-bipyridyl and 1,10-phenanthroline, respectively. The difference in CSS band bending, $\Delta\nu$ =44 and 77 for complex-1 and 2 shows that dithioate moiety act as monodentate. The peaks at 555 cm^{-1} for Cu-N, and 426 cm^{-1} for Cu-S confirm the complex formation.
- The elemental analysis shows a good agrrement between the found and calculated values that helps in the conformation of the complexation.
- The Cu(II) 4-(4-nitrophenyl)piperazine-1-carbodithioate complexes interact directly with CT-DNA, leading to disfunctioning and cleavage of the macromolecular structures. The values of intrinsic binding constant (K_b) calculated for these complexes are very high as compared to the standard, this may be due to sulfur donar ligand and their confirmation due to the benzene rings.
- The Cu(II) 4-(4-nitrophenyl)piperazine-1-carbodithioate complexes also show good result as antioxidant against DPPH free radicals. The IC_{50} calculated for these complexes are very low and comparable to that of standard Ascorbic acid. The IC_{50} valve for complex-1 is low means highly effective as compared to complex-2, this may be due to the easily confirmation on 2,2-bipyridyl ligand as compared to 1,10-phenanthroline.
- The Cu(II) 4-(4-nitrophenyl)piperazine-1-carbodithioates show pH dependant redox behavior and confirmed by cyclic voltammetry results. From their mechanisms, it has been concluded that mostly reduction and oxidation involves one electron one proton, while in acidic medium (pH=3), two electrons and two protons are involved during forward scanning (oxidation). From the study of voltammograms, it is also deduced that here proton involved in oxidation and reduction process.