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Synthesis and studies of Cu(II)-thiolato complexes: bioinorganic perspectives

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Contents

Abstract

Ligation of thiolate sulfur to copper at the active sites of quite a number of copper proteins has been established either by X-ray crystallographic and/or by spectroscopic studies. In addition, for Cu(II)-substituted metalloproteins, the presence of Cu(II)-thiolate bonding at the active sites could be established spectroscopically. Cu(II)-thiolate bonding in different enzymes is not always very similar. Obviously, the bioinorganic significance of Cu(II)-thiolate bonding is enormous and has attracted a lot of attention to synthesize model Cu(II)-thiolato complexes as electronic structural analogues of the active sites of these biomolecules. The present review deals with (i) nature of Cu(II)-thiolate bonding present in different metalloproteins, (ii) difficulties involved in the synthesis of Cu(II)-thiolates and ways to surmount them, (iii) characterization of the Cu(II)-thiolate bonding by electronic and EPR spectroscopic techniques and (iv) electron transfer properties of the Cu(II)-thiolato complexes by cyclic voltammetric studies.

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The properties of the Cu(II)-thiolato complexes have been discussed as possible models for the active site(s) of copper proteins. © 1997 Elsevier Science S.A.

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1. Introduction

Numerous metalloproteins contain one or more copper atoms at the active site. These proteins are involved in a wide variety of biochemical functions [1–3] which depend upon the variation in the chemical nature and geometrical disposition of the ligation at the active sites. The use of spectroscopic techniques to understand the electronic structure and bonding of these active sites is a subject of continual interest [4–7]. These studies are being made with the ultimate aim to understand the biochemical functions of the metalloproteins at the molecular level. However, the metal binding sites in many of these proteins are intrinsic [8] in nature, i.e., they are formed as a result of intimate interactions of the copper ions with the amino acid residues of the proteins. An intrinsic active site generates an unusual coordination environment at the metal binding site(s) due to conformationally imposed constraints upon the amino acid residues that provide ligation to the metal ion(s). Thus, an unusual coordination is imposed on the metal ion at the active site which is a design by nature to effect a condition energetically favourable to carry out its assigned biological role(s). However, this leads to two important problems: (i) spectroscopic studies on the native proteins yield results that are difficult to interpret and (ii) it is almost impossible to synthesize a small molecular weight complex which can serve as a detailed model of the active site. It is, therefore, necessary to synthesize and study small molecular weight, well-characterized model complexes that can duplicate specific protein features and hence can provide a data base to aid in the interpretation of observations on the native proteins.

This review aims to survey the copper(II)-thiolate complexes with ligands containing at least one thiolate sulfur donor in addition to other donor atoms such as nitrogen, oxygen and thioether sulfur. The literature coverage is approximately upto the beginning of 1996. These complexes are discussed with a bias towards their bioinorganic significance. This is because most of these studies have been made as possible models for the active sites of a number of copper proteins. The complexes with 1,1- or 1,2-dithio ligands e.g., xanthate, thioxanthate, dithiolene, etc. or their monothio analogues have not been included since their structural and electronic properties are often largely a result of the characteristic π-delocalization onto the ligand moiety [9]. Moreover, none of these ligands has been found to be of biological relevance as far as copper containing metalloproteins are concerned.

The review is divided into several sections. The first section deals with the study of Cu(II)-thiolate bonding present in several metalloproteins. The second section discusses the synthetic approaches to Cu(II)-thiolate compounds. Finally the third section deals with the spectroscopic as well as electrochemical studies of the model Cu(II)-thiolates and their relevance to the in vitro studies on copper metalloproteins. The final section of this review deals with conclusion and future scope.

2. Copper thiolate bonding in copper proteins

The ligation of thiolate sulfur to copper at the active sites of quite a number of copper proteins has been established. Several excellent books/reviews deal with the copper proteins [1–7]. Copper(II)-thiolate bonding is shown to be present by X-ray crystallography in the case of single copper proteins such as plastocyanin [10], azurin [11], basic blue protein from cucumber (CBP) [12], bacterial blue protein [13], amicyanin [14] from *T. versatur* and in the multi-copper protein, ascorbate oxidase [15]. In the case of other mono-copper blue proteins such as stellacyanin [16], rusticyanin [17] or multicopper oxidases [18] such as tree and fungal laccases, ceruloplasmin [19] etc., copper-thiolate bonding has been established by various spectroscopic studies. Non-blue proteins like the Cu_A site in cytochrome C oxidase [20] and nitrous oxide reductase [21] also contains two cysteine thiolates bonded to Cu(II). In addition, Cu(II) ion has been used [22] as a spectroscopic probe for the active sites of different metalloproteins. In these cases as well, the presence of Cu(II)-thiolate bonding at the active sites can be established spectroscopically. Thus, the bioinorganic significance of Cu(II)-thiolate bonding is enormous. Particularly, the blue protein active sites (type 1 site) have spectroscopic signatures [4,5] attributable to a significant extent to the presence of strong Cu(II)-thiolate bonding. Note that Canters and coworkers carried out site-directed mutagenesis on *P. aeruginosa* replacing the residue Met44 by a lysine [23]. This had a dramatic effect on its function as an electron transfer enzyme.

Of all the mononuclear Cu-proteins, plastocyanin is the one subjected to the most number of studies [5,24] and hence is the best documented metalloprotein. The X-ray structures [25,26] of poplar plastocyanin in both +2 and +1 oxidation states have been determined. The structure in the oxidized state at pH 6.0 has been determined to a resolution of 1.6 Å while at pH 4.2 the resolution was 1.9 Å. The coordination geometry of the copper atom is distorted tetrahedral with two normal Cu(II)-N(His) and one strong Cu(II)-thiolate(Cys) bonds (Table 1). The fourth bond between the Cu and a thioether sulfur (Met) is very long. Thus, the copper ion has (3+1) coordination and the coordination geometry is more like flattened

Table 1
Bond distances (Å) and angles (°) at the Active site of poplar plastocyanin [25,26]

	pH 6.0	pH 4.2
Cu–N (His 37)	2.04	2.07
Cu–N (His 87)	2.10	2.17
Cu–S (Cys 84)	2.13	2.11
Cu–S (Met 92)	2.90	2.87
N (His 37)-Cu–N (His 87)	97	96
N (His 37)-Cu–S (Cys 84)	132	133
N (His 37)-Cu–S (Met 92)	85	87
N (His 87)-Cu–S (Cys 84)	123	122
N (His 87)-Cu–S (Met 92)	103	103
S (Cys 87)-Cu–S (Met 92)	108	109

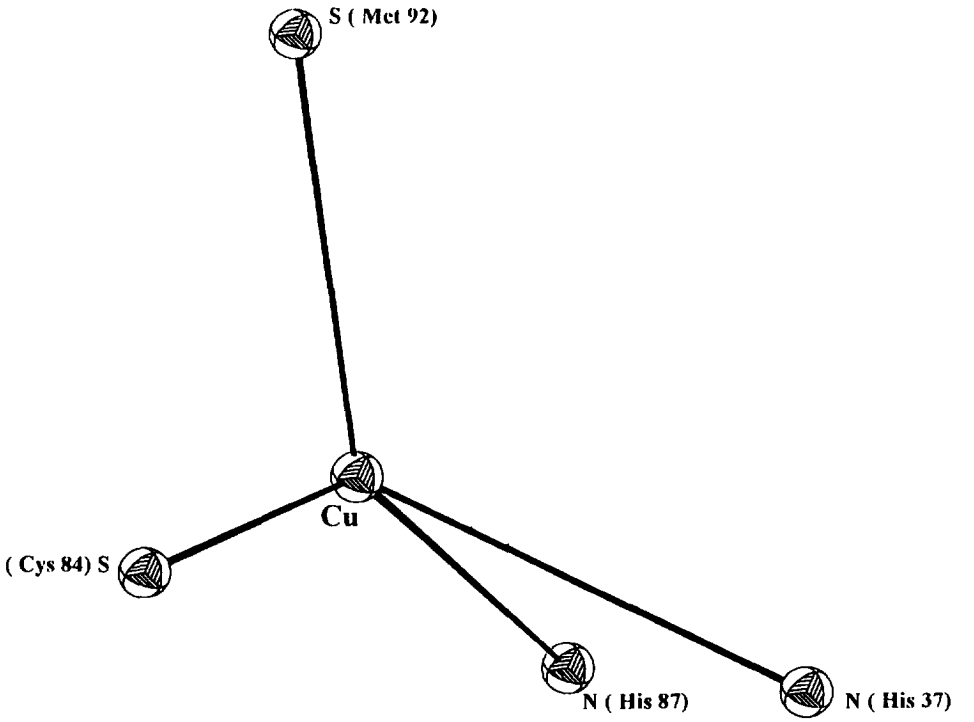


Fig. 1. An illustration of the active site in poplar plastocyanin.

tetrahedral. This geometry around the metal ion is not affected between pH 6.0 and 4.2. Fig. 1

EXAFS studies [27] of Cu(II)-plastocyanin in solution and in crystals have given values which are in close agreement with the Cu-N and Cu-S(Cys) bond distances found from X-ray crystallography. However, the Cu-S(Met 92) length was not detected by the EXAFS studies.

The crystal structure of plastocyanin from a green Alga *Enteromorpha prolifera* has also been determined [28]. The copper sites of this protein and of plastocyanin from poplar have closely similar structures. The largest bond length difference occurs at Cu-N(His 37) which is about 0.15 Å longer in the latter. However, significant differences exist at sites remote from the active site.

The structure of Cu(II)-azurins from three sources viz. *A. denitrificans* [29], *P. aeruginosa* [30] and *P. denitrificans* [31] has been determined to 1.8, 2.7 and 3.0 Å resolutions respectively. The overall structure of the active site is essentially the same in the three sources. For the structure at 1.8 Å resolution [29], the coordination geometry is different from that present in the Plastocyanin active site, and can best be described as distorted trigonal bipyramidal with strong in-plane bonds to two Cu(II)-N(His) and one Cu(II)-thiolate(Cys) (Table 2). In this (3+2) coordination set-up, the two axial positions are occupied by one thioether S(Met) and one O(Gly).

Table 2
Bond distances (Å) and angles (°) at the Active site of Azurin from *A. denitrificans* [29]

	Molecule 1	Molecule 2
Cu–O (Gly 45)	3.16	3.09
Cu–N (His 46)	3.08	2.09
Cu–S (Cys 112)	2.12	2.17
Cu–N (His 117)	2.01	1.99
Cu–S (Met 121)	3.12	3.10
O (Gly 45)-Cu–N (His 46)	72	76
O (Gly 45)-Cu–S (Cys 112)	103	104
O (Gly 45)-Cu–N (His 117)	78	81
O (Gly 45)-Cu–S (Met 121)	146	148
N (His 46)-Cu–S (Cys 112)	135	135
N (His 46)-Cu–N (His 117)	101	108
N (His 46)-Cu–S (Met 121)	79	75
S (Cys 112)-Cu–N (His 117)	122	116
S (Cys 112)-Cu–S (Met 121)	109	105
N (His 117)-Cu–S (Met 121)	94	98

The unit cell contains two independent molecules with slightly different geometries in the asymmetric unit Fig. 2. The 2.0 Å resolution structure [32] of the blue protein cupredoxin from *Alcaligens faecalis* S-6 show that Cu(II) is bound to four donors in a distorted tetrahedral fashion (Fig. 3) Table 3. Although the bond distances are not very accurate at this stage, that the Cu(II)-S(Met) distance is much shorter than that found in plastocyanin and azurin.

In the case of CBP, the structure [33] around Cu(II) is distorted tetrahedral with coordination from N(His 39), N(His 84), S(Cyst 79) and S(Met 89). However, further refinement will be required before bond length and bond angles at the Cu-atom can be stated with confidence. At this stage, there is no evidence for a fifth Cu-ligand bond.

Out of all the blue copper oxidases, only the crystal structure of ascorbate oxidase is available [34]. It consists of a mononuclear blue site and an unprecedented trinuclear copper site. The trinuclear site does not have Cu(II)-thiolate ligation. In the blue site, the Cu(II) ion is coordinated (Table 4) to two histidine imidazoles, one cysteine thiolate and one methionine thioether as in plastocyanin. Two subunits are present in the asymmetric unit.

A careful look at the active site structures of blue copper proteins reveal an important fact: in all cases, the Cu(II)-S(Cys) bond distance is shorter than that obtained with model complexes (*vide infra*). This strong Cu(II)-thiolate bonding along with unusual coordination geometry is reflected in the peculiar spectroscopic properties of the blue site.

Besides these type 1 proteins, Cu(II)-thiolate bonding is present in the Cu_A site in cytochrome c oxidase [20]. X-ray absorption edge [35]a EXAFS [35]b, EPR [35]c and ENDOR [35]d studies indicated that the Cu_A site was a pseudotetrahedral $CuN_2(his)S_2$ (cys) unit with considerable Cu(I)-thiyl radical or unusually covalent Cu(II)-thiolate character (Fig. 4). However, recent X-ray crystallographic studies

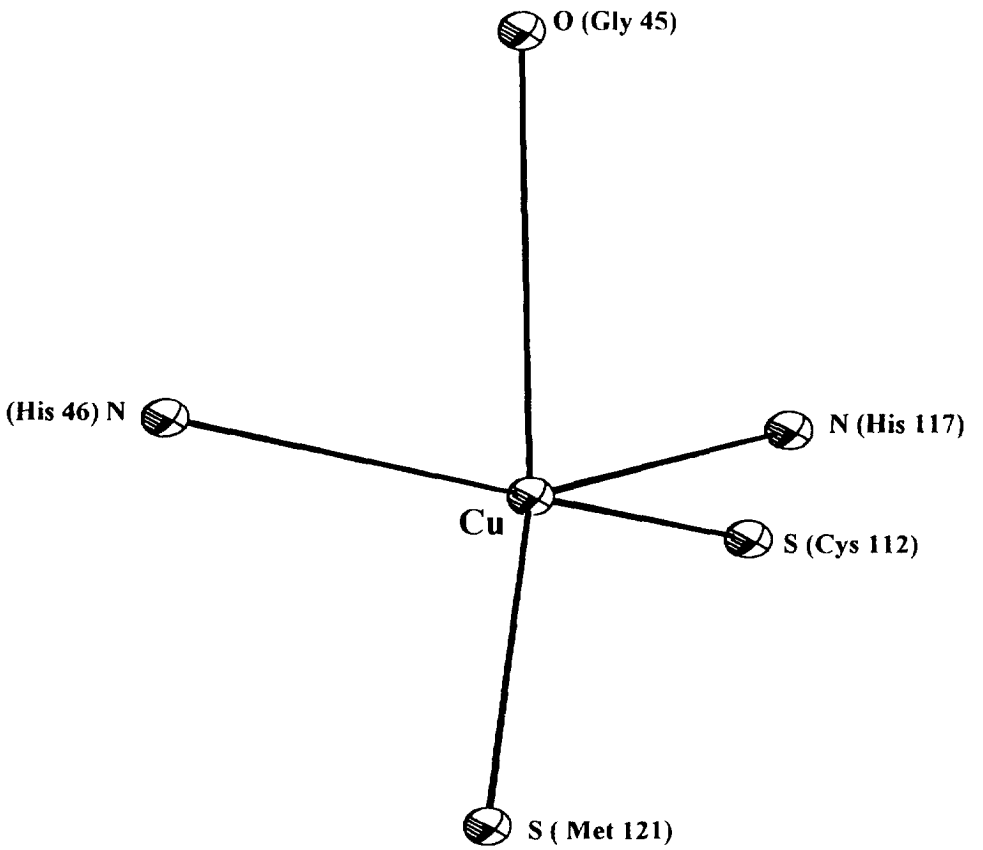


Fig. 2. An illustration of the active site in azurin from *A. denitrificans*.

have firmly established that this site has a dicopper unit ligated by 2 cysteine thiolates, 2 histidine imidazoles and one methionine in an unknown arrangement [36]. A similar ligand environment has also been suggested to be present at the active site of nitrous oxide reductase [37].

A few other non-copper proteins contain thiolate ligation at the active sites. In these proteins, Cu(II) ion has been substituted for the metal ion present in the native enzymes as a spectroscopic probe and the presence of thiolate bonded to the metal ion(s) at the active sites has been shown. One such example is Cu(II)-substituted horse LADH [22].

2.1. Thiolate as a ligand to transition metal ions

Sulfur is less electronegative than oxygen or nitrogen. As a result, sulfur exhibits markedly different bonding properties compared to nitrogen or oxygen. Also, sulfur being a member of the third period, has low lying empty 3d orbitals. These orbitals have been implicated in the many differences observed in its properties when com-

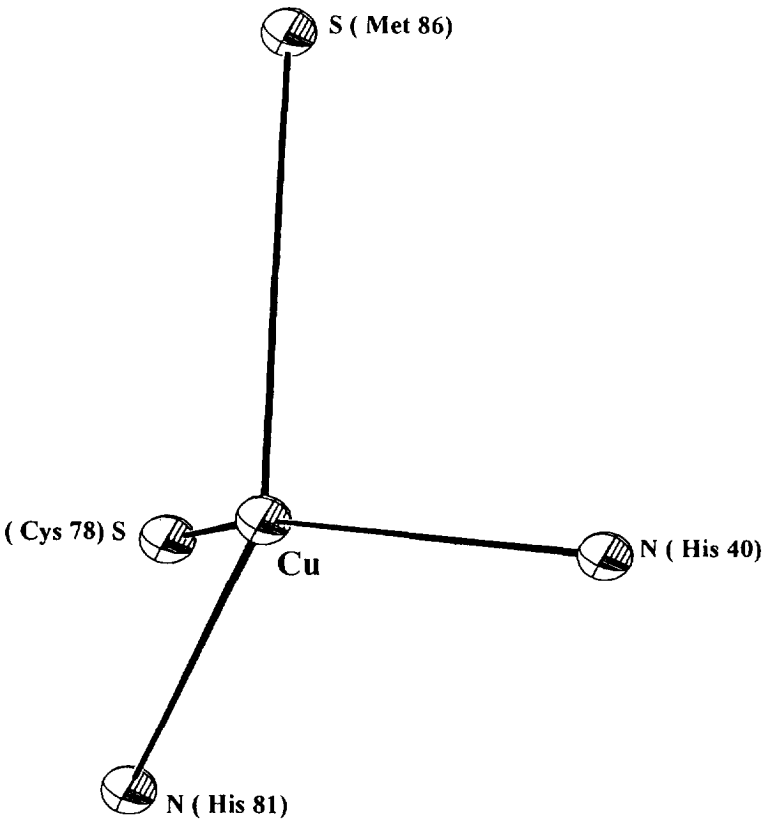


Fig. 3. An illustration of the active site in present in cupredoxin from *alcaligens faecalis S-6*.

Table 3
Bond distances (Å) around Cu in *A. faecalis* [32]

Cu–S (Cys 78)	2.07
Cu–N (His 40)	2.10
Cu–N (His 81)	2.21
Cu–S (Met 86)	2.69

Table 4
Bond distances (Å) around Cu in ascorbate oxidase [34]

	Molecule A	Molecule B
Cu–N (His 445)	2.10	2.12
Cu–N (His 512)	2.05	2.11
Cu–S (Cys 507)	2.13	2.03
Cu–S (Met 517)	2.90	2.83

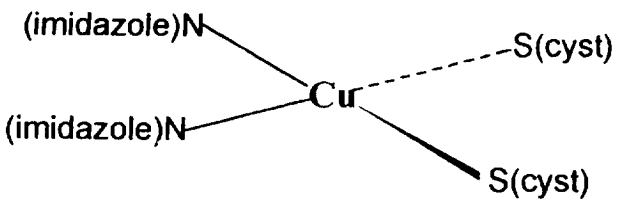


Fig. 4. The proposed Cu_A site in cytochrome C oxidase.

pared to O and N donors. The geometry of coordination around thiolato S donors compared to O and N donors is also important e.g. S often adopts tetrahedral rather than trigonal coordination. A large number of reviews describing different examples of metal thiolate complexes is evidence of increasing interest in the interaction of thiolate ligands with metal ions [38–44]. The involvement of metal thiolates in the catalysis of biological [45] and non-biological [46] processes reflects the multifaceted chemistry of metal thiolate bonds. The observation that the ligating power of sulphur in high oxidation states is less effective suggests [47] that the origin of the ligating power is not the availability of 3d-orbitals on sulphur, but rather is a consequence [48] of the high polarizability of sulphur. However, it is most likely that both factors are operational in metal-thiolate chemistry.

The synthesis of thiolato complexes is not trivial, but is hampered by difficulties [38,39] inherent in thiolate chemistry. These are:

(1) Thiolates frequently act as bridging ligands. This is a potential hurdle to the synthesis of mononuclear metal thiolates.
(2) Thiolates show a strong tendency to act as reducing agents with the formation mostly of disulfides, which has hampered synthesis of complexes of metals in their higher oxidation states. This is particularly so in the case of Cu(II), Fe(III) etc.
(3) In coordinated alkane thiolates, C–S bond cleavage may readily occur giving rise to the formation of a number of products.

2.2. Synthesis of copper(II)-thiolate complexes

The interest of coordination chemists in the intriguing natural systems has led to many attempts to create models for the type 1 copper sites by synthesizing complexes containing copper(II)-mercaptide linkages. This is a challenging synthetic problem due to the facile redox reaction which normally converts Cu(II) and mercaptide sulfur to copper(I) and a disulfide [49–51]. Uncertainties exist regarding the nature of the Cu(I) species although this is not of concern to the present review. Besides, comparatively little is known about the kinetic and thermodynamic stabilities of the RS^--Cu(II) bond in solution as the internal electron transfer is quite fast in these systems. However, quite a few kinetic studies have been carried out with different mercaptans to evaluate quantitatively, the relative contributions of coordination geometry, electronic and steric characteristics of ligand donor atoms, as well

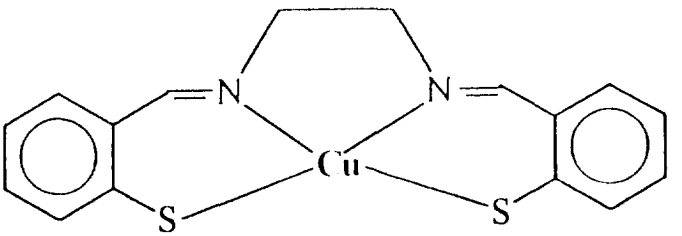


Fig. 5. The structure of cis-CuN_2S_2 complex.

as the Cu(II)/Cu(I) potential to Cu(II)-thiolate bond stability. These studies point [50,51] to the fact that reduction of Cu(II) by mercaptans is mostly second order with respect to inner-sphere S-bonded intermediates leading to concerted two-electron transfer and formation of S–S bond. On the other hand, a unimolecular pathway can be proposed [52] in complexes where the oxidizing strength of the Cu(II) centre permits the formation of metal stabilized thiyl or disulfide radicals.

Earlier attempts in synthesizing Cu(II) thiolato complexes have generally resulted in unstable [53,54] and/or incompletely characterized [55,56] materials. Australian workers synthesized and X-ray crystallographically characterized [57] the first Cu(II)-thiolato complex in 1977. The ligand used was a tetradentate N_2S_2-donor derived from Schiff base condensation of o-mercaptobenzaldehyde with ethylenediamine. The structure (Fig. 5) consists of monomeric cis-CuN_2S_2 units. While full details of the structure are still not published, the EPR spectrum of the complex shows rhombic splitting which was suggested to be due to a geometry somewhat distorted from planarity. However, the EPR rhombicity for this complex need not imply non-planarity of the coordination geometry since the cis-geometry is already non tetragonal. Complete electronic spectral data are also not yet available and copper centred bands might be obscured by strong near-UV absorptions of the ligand itself. Since that time, quite a few Cu(II)-thiolato complexes have been synthesized following different strategies.

One such strategy involves anchoring a Cu(II) ion in a suitable macrocycle or a polydentate N-donor ligand before introduction of the thiol to the synthetic mixture. The extremely stable and relatively inert complex thus formed shifts the Cu(II)/Cu(I) potential to the negative side making it more difficult to reduce. Moreover, the macrocycle/polydentate N-donor ligands blocks all coordination sites except the site to be used by the thiolate ligand, thus preventing the dimerization step. Following this strategy, a number of Cu(II)-thiolates have been isolated [58–63] and characterized in the solid state. A stable Cu(II)-thiolate formed in the reaction of the macrocyclic tetramine complex, $[Cu(tetb)]^{2+}$ with o-mercaptobenzoate has [58] approximately trigonal bipyramidal geometry (Fig. 6) where the thiolate sulfur occupy an equatorial position. For the equatorial donor set (SN_2), the two N-atoms come from the folded macrocycle. The observed Cu(II)-thiolate bond length (2.359 Å) is slightly longer compared to planar CuN_2S_2 systems. Two approximately square pyramidal CuN_4S complexes have been reported [59]

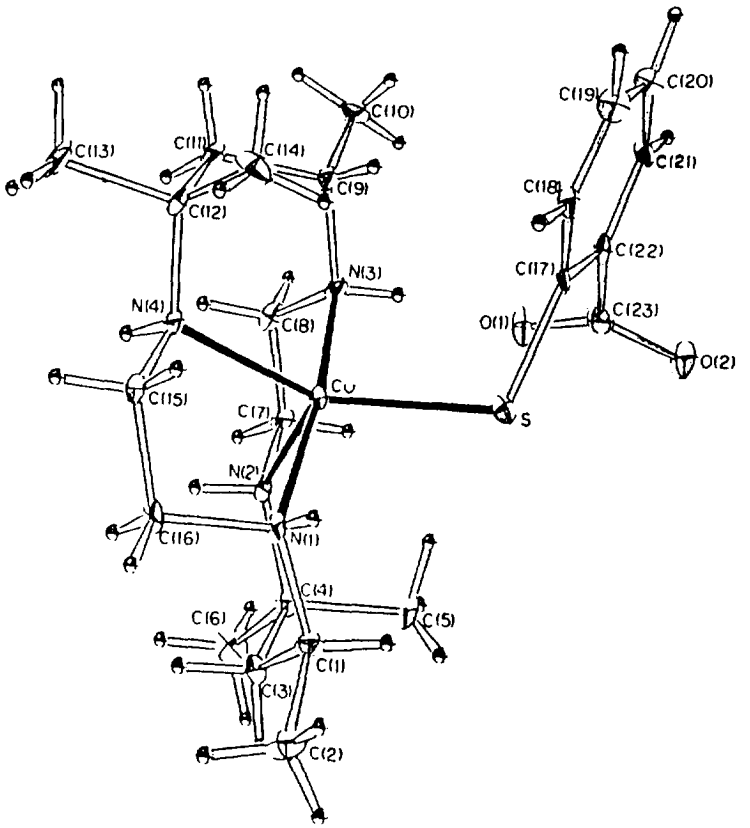


Fig. 6. The structure of [Cu(tetb) (o-mercaptobenzoate)]. H_2O.

where the four equatorial N-donors are supplied by the monoanionic tetramine ligand shown in the Fig. 7a and the apical S-donor is either thiophenolate or p-chlorophenolate. The average Cu(II)-thiolate bond distance (2.456 Å) is somewhat longer than that observed in the case of [Cu(tetb)(o-mercaptobenzoate)] [58]. However, considering its axial nature, the Cu(II)-thiolate bond should be considered as strong. Japanese workers reported [60] an interesting thiolate bridged Cu(II) dimer in which a single p-methylbenzene thiolate serves as the apical ligand for two CuN_4 units similar to the one shown in the Fig. 7 except that the two oxime oxygens are linked (Fig. 8) with a BF_2-fragment. Robson and coworkers have also reported thiolate bridged dinuclear Cu(II) complexes [62]. All of the structurally characterized complexes described above contain ligation by aromatic thiolates. Aliphatic thiolates also give similar complexes although they are less stable. The higher stability of aromatic thiolates is attributed to the delocalization of charge from S to the aromatic ring. With $[Cu(cyclam)](ClO_4)_2$, isopropyl mercaptan forms a square pyramidal CuN_4S complex [61] in the solution phase. This complex has not been characterised in the solid state. A green coloured trigonal bipyramidal complex of

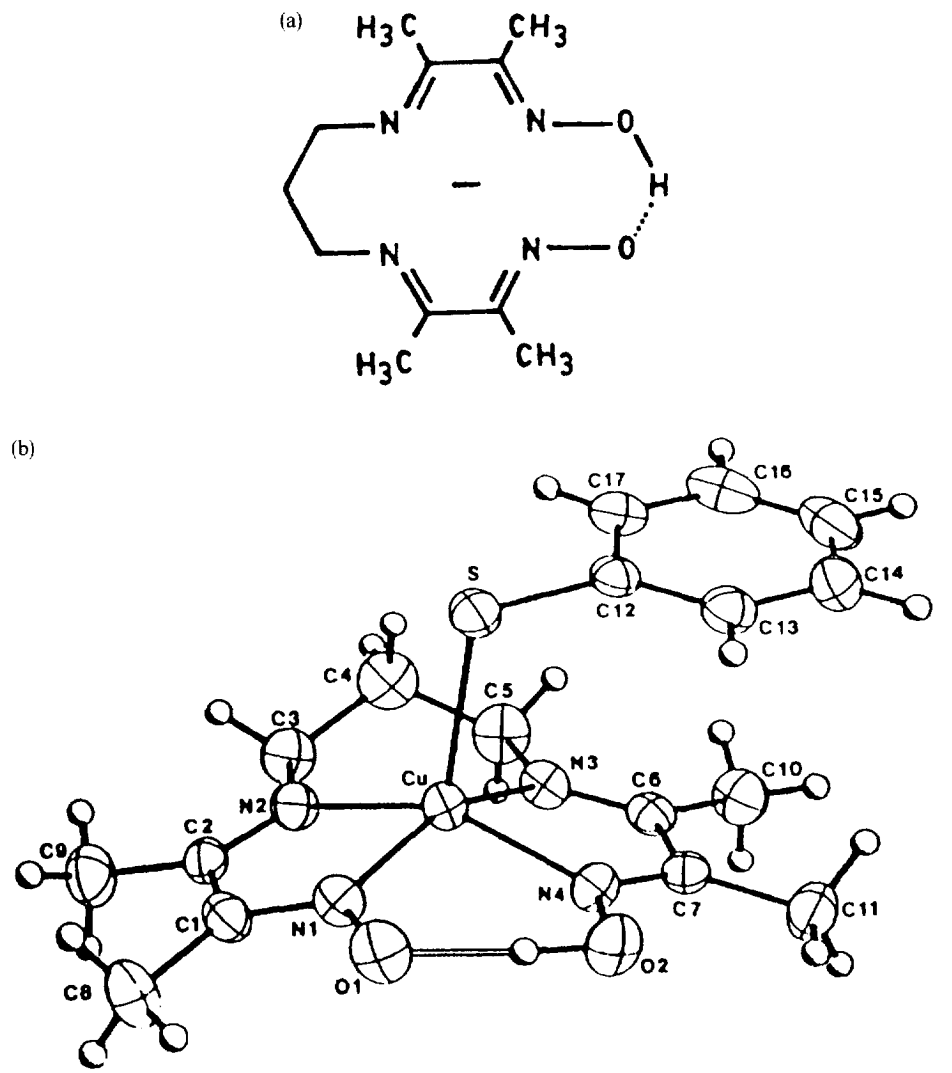


Fig. 7. (a)The monoanionic tetramine ligand. (b) The square-pyramidal structure with thiophinolate.

$[Cu(tetb)]^{2+}$ and 3-mercaptopropionate has been characterized [63] in the solid state (Fig. 9). The equatorial Cu(II)-S distance (2.314 Å) is shorter than that reported for the aromatic thiolates. With mercaptoacetate, $[Cu(tetb)]^{2+}$ forms a complex [64] which is a rarely documented example of a ligating hydropersulfide (Fig. 10). The coordination geometry around Cu(II) is, however, similar to that present in the complex with 3-mercaptopropionate.

Another strategy of synthesizing Cu(II)-thiolates involve reacting the Cu(II)-complex of the trispyrazolylborate ligand (Fig. 11). This ligand was chosen to enforce a nonplanar, trigonal geometry on to Cu(II) so that the vacant site can be

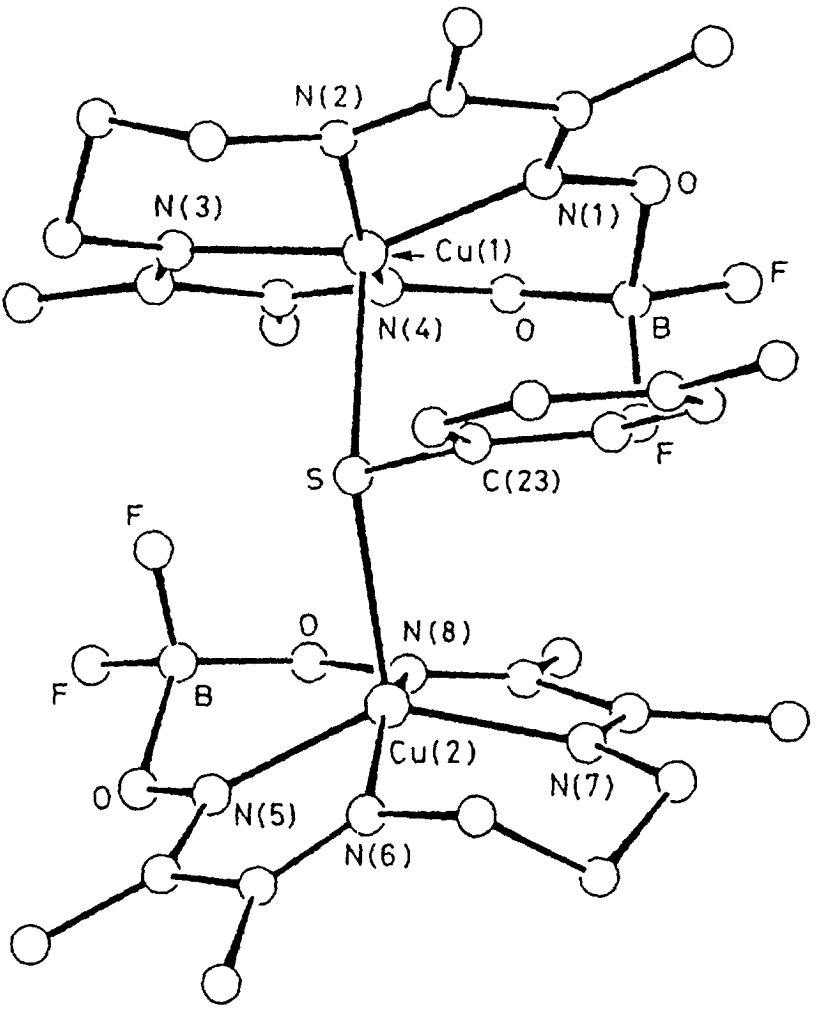


Fig. 8. The structure of $[Cu(cyclops)_2(SC_6H_4\text{-}p\text{-}Me)](ClO_4)$.

occupied by a suitable thiol giving a pseudotetrahedral Cu(II)-thiolate complex with the chromophore CuN_3S. The choice of this ligand was based on the fact that the three nitrogen atoms of the ligand have been shown [65] to donate appreciable electron density to the copper ion and hence should shift the Cu(II)/Cu(I) potential towards the negative side making it difficult to reduce by the thiol. This strategy has been used [66] to prepare the following pseudotetrahedral complex (Fig. 12). When the sterically constrained tris-pyrazolyl borate ligand was used the dimerisation pathway could be blocked which made possible the synthesis of aliphatic thiolates [67] though in the solution state. Only recently, one complex has been X-ray crystallographically characterised [68] in this category. Kitajima et al. reported the structure of $[Cu(HB(3,5\text{-}iPr_2pz)_3(SCPh_3)]$ with the $Cu(II)N_3S$ chromophore. The

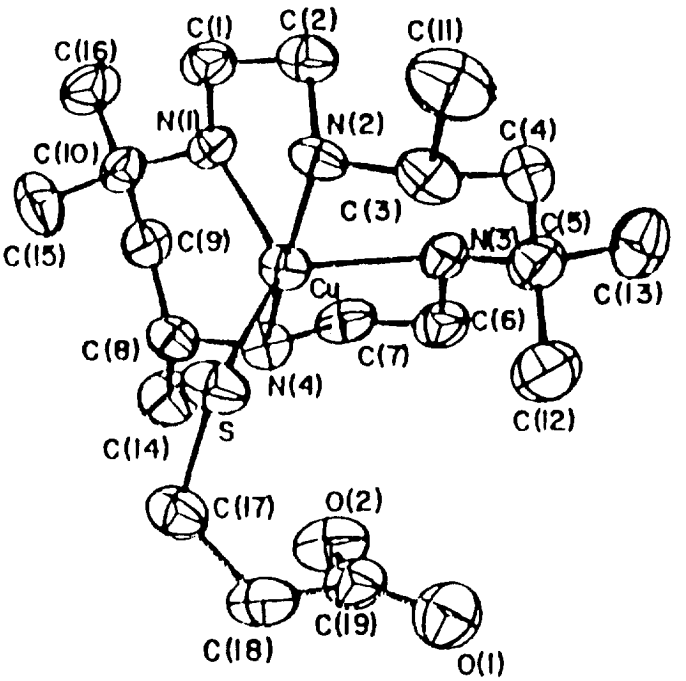


Fig. 9. The structure of [Cu(tetb) ($SCH_2CH_2CO_2$)].2CH_3OH.

monomeric structure (Fig. 13) is best described [68] as trigonal pyramidal. The basal plane consists of two N and the thiolate sulfur. The Cu(II) ion is about 0.20 Å above the basal plane. The Cu(II)-S bond distance (2.12 Å) is shorter than that of other model complexes but compares well with the Cu(II)-thiolate bonding present at the blue site.

Multidentate ligands incorporating thiol(s) have been used to complex Cu(II) with the idea that the resulting complexes would be stable both thermodynamically and kinetically due to formation of multi-chelated structures. Thus, the tripeptides, N-mercaptoacetylglycyl-L-histidine (MAGH) and N-mercaptoacetyl-DL-histidyl-DL-histidine (MAHH) produce metastable square-planar and square-pyramidal Cu(II) complexes respectively [69]. Similar square-planar copper(II) complexes of peptides such as N-mercaptoacetyl-L-histidine (MAH) and 2-mercaptopropionyl-L-cysteine (2-MPC) have been previously reported in solution [70]. A green coloured α-mercptopropionylglycine-Cu(II) complex which involves thiol, neighbouring deprotonated peptide nitrogen, terminal carboxylate and oxygens from water as the coordinating atoms has been reported [71]. Other thiols e.g., 2-mercaptoethylamine give similar [72] but short-lived species, and in one case a flow system has been employed [50] with epr spectroscopy to characterize transient ternary complex formed from copper(II), glycylglycine and cysteine. It must be realized that these are not well characterized species and any conclusion regarding structure and bonding must be made with caution. To model the type 1 site with the amino acid

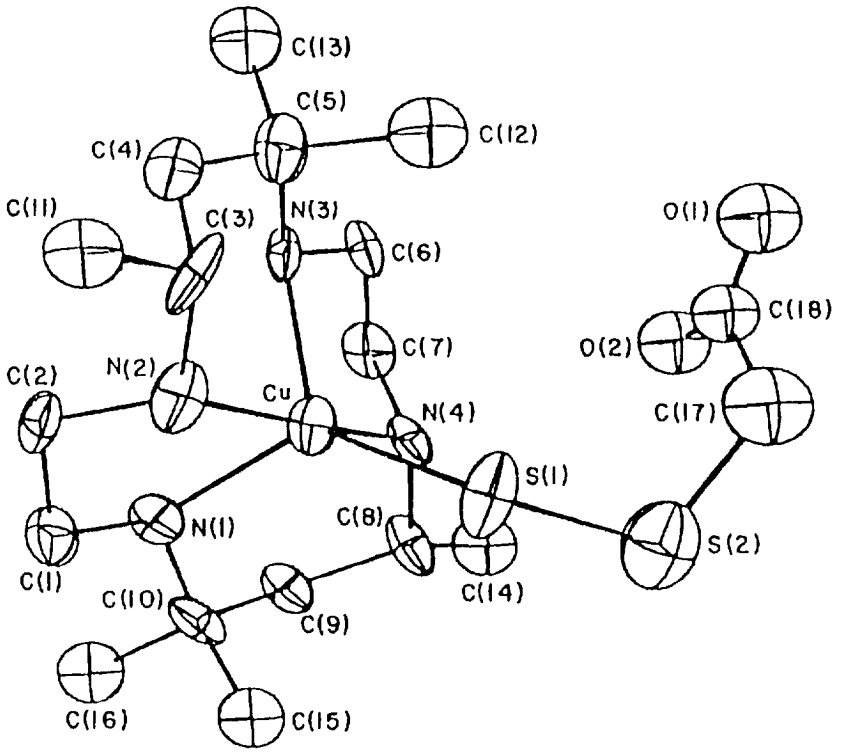


Fig. 10. The structure of [Cu(tetb) ($SSCH_2CO_2$)].$2CH_3OH$.

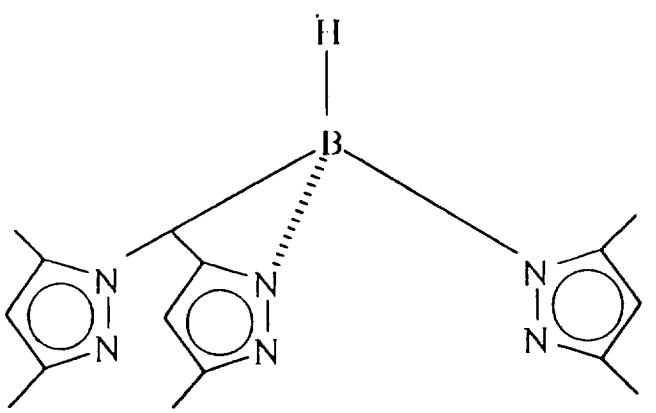
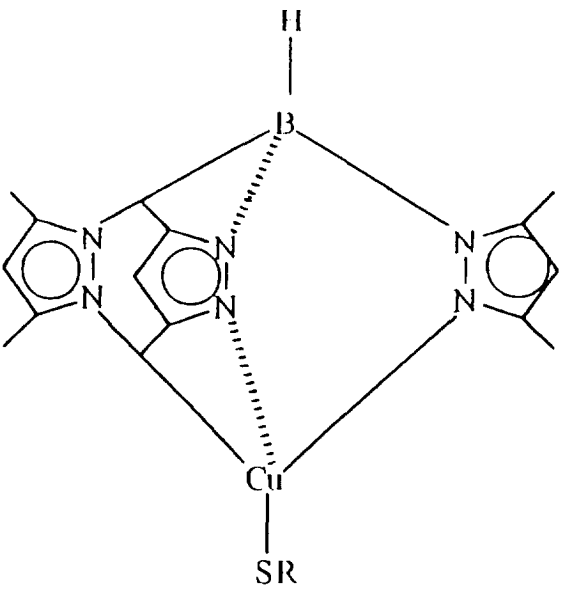


Fig. 11. A tris-pyrazolylborate ligand.

cysteine, it was realized that when two cysteine units are connected by an ethylene linkage through the amino groups, they can act as a potential tetradentate ligand with the N_2S_2 chromophore. This has actually found to be so. Thus, when



SR = p-NO_2 thiophenolate, O ethylcysteinate, etc.

Fig. 12. Psedotetrahedral CuN_3S complexes with tris-pyrazolylborate.

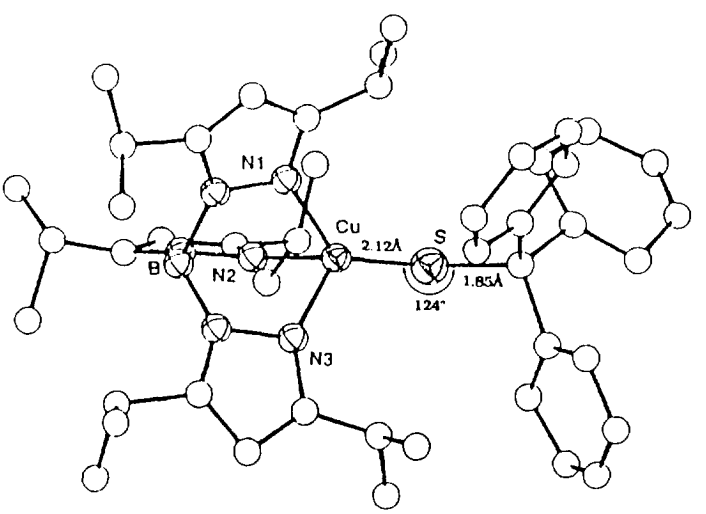


Fig. 13. The structure of $[Cu(HB(3, 5\text{-}iPr_2pz)_3)(SCPh_3)]$.

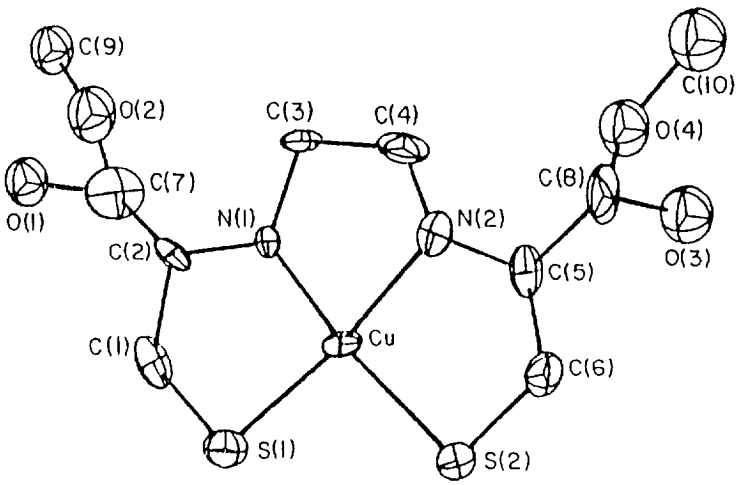


Fig. 14. The structure of [Cu(SCH$_2$CH(CO$_2$Me) NHCH$_2$-)-$_2$].

[Cu(tetb)]$^{2+}$ was allowed to react with the ligand at room temperature in methanol in presence of NaOH, the colour of the solution slowly changed to dark red with the formation of square planar, anionic cis-CuN_2S_2 complex and solid tet-b precipitated out quantitatively. This complex could be isolated [73] in the solid state as a neutral complex when the two carboxylic acid groups were esterified with methanol. The structure (Fig. 14) is almost planar with a small (21°) pseudotetrahedral twist. The Cu(II)-thiolate average bond distance of 2.246 Å is, however, longer than that in plastocyanin (2.13 Å). The high stability of this complex formation drives the metal ion out of the macrocycle tet-b. Three 5-membered chelate rings along with the tendency of Cu(II) to form square planar geometry makes this complex remarkably stable. Anchoring the Cu(II) ion inside the macrocycle stabilizes the Cu(II) state and effectively inhibits the formation of the Cu(I) species. However, when $[Cu(en)_2](ClO_4)_2$ was used as the starting material, partial reduction of Cu(II) to Cu(I) cannot be prevented and a novel pentanuclear species could be isolated [74] and characterized in the solid state (Fig. 15). The structure consists of three Cu(I) ions connected to two cis-CuN_2S_2 units in such a way that each Cu(I) is bonded in a trigonal planar fashion to three sulfur atoms. Interestingly, it was long known [75] that Cu(II) and D-penicillamine forms an intensely purple solution at physiological pH. Freeman and Birker isolated [76] from this purple solution, an anionic cluster complex, $[Cu(II)_6Cu(I)_8(D\text{-}Pen)_{12}Cl]^{5-}$. This anion crystallizes with $[Co(NH_3)_6]^{3+}$, $[Ru(NH_3)_6]^{3+}$ and Tl^+ as counterions. The X-ray structure of the complex with Tl^+ as the counterion shows eight tetracoordinated Cu(I) ions with ligation from three S and a central Cl^- ion. Each Cu(II) ion is also tetracoordinated to two thiolate S and two amino nitrogen atoms of the two penicillaminate ligands to which the sulfur atoms belong. Another closely similar structure with Cu(II)-thiolate bonding has been characterized [77] with D-penicillamine with the formulation, $[(Cu(I)_8(Cu(II)_6(SC(CH_3)_2CH_2NH_2)_{12}Cl].\sim 3.5SO_4.\sim 19H_2O$. The six Cu(II)

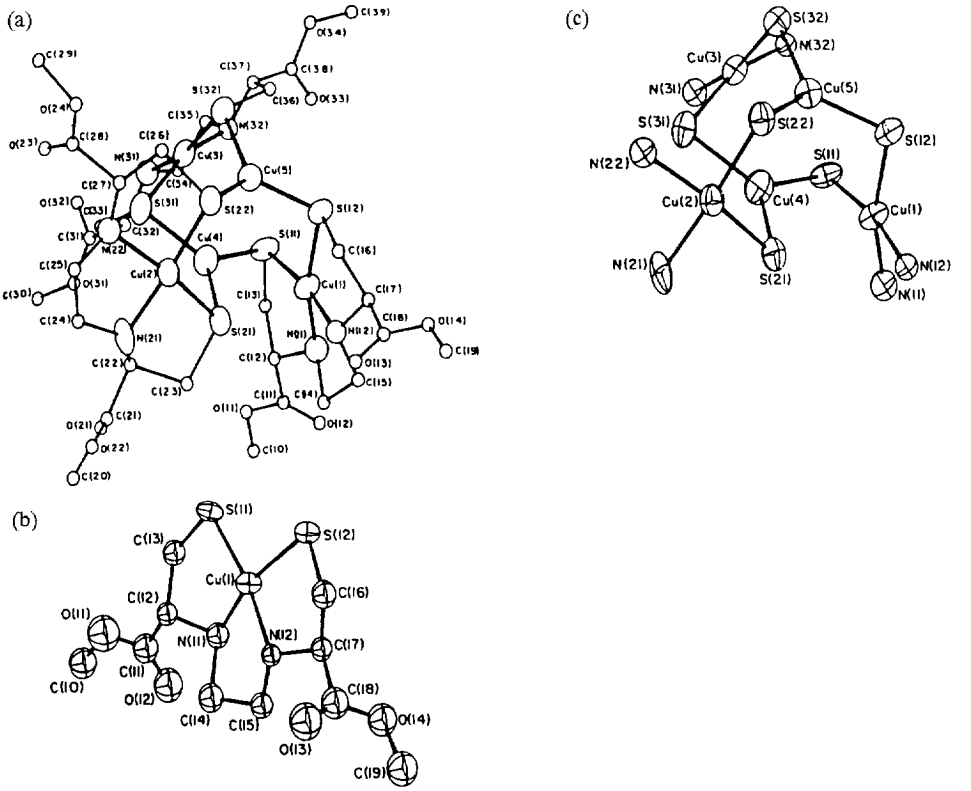


Fig. 15. (a) The structure of the mixed-valent pentanuclear Cu-complex, (b) View of one of the Cu(II) N_2S_2 unit, (c) View of the $Cu_5S_6N_6$ cluster framework.

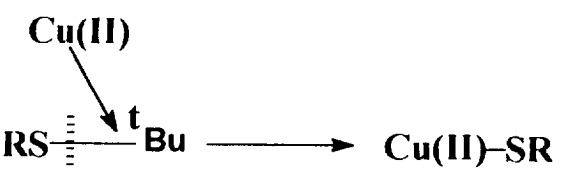


Fig. 16. Breaking of S-^{t}Bu linkage by Cu (II).

ions have an approximately planar cis-CuN_2S_2 chromophores. The average Cu(II)-thiolate bond distance of 2.28 Å is quite similar to the one found in the structure reported by Freeman and Birker. Synthesis of mixed-valent Cu(II)-Cu(I) thiolate compounds have assumed major importance after the reports of the X-ray crystallographic studies on cytochrome c oxidase and nitrous oxide reductase [36,37]. The active site in these two enzymes consists of a dicopper unit with cysteine thiolate bridging. Tolman and coworkers have reported an interesting dicopper system with thiolate bridging to model the active sites of the above two enzymes [78].

The interesting synthetic strategy of Becher et al. [79] makes use of Lewis acidity

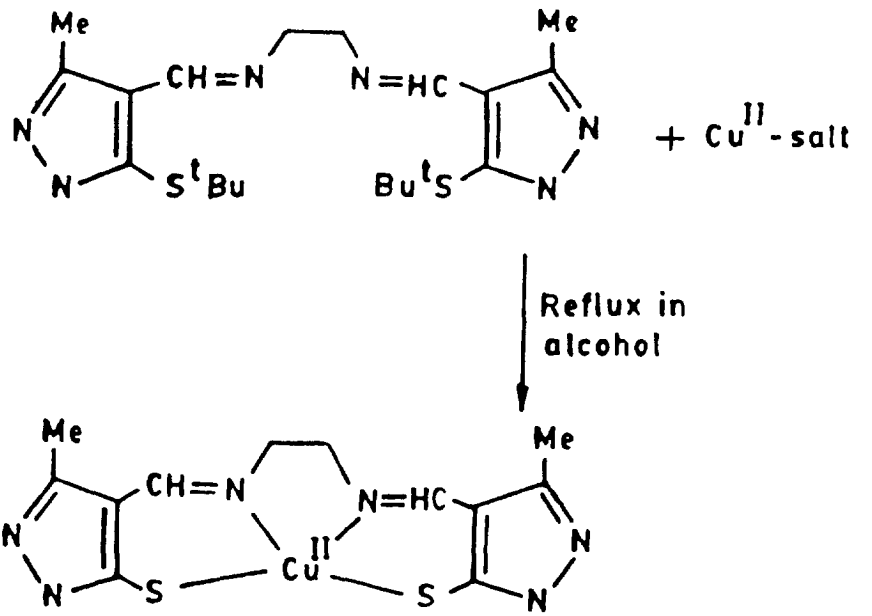


Fig. 17. A typical cis-CuN_2S_2 complex synthesized by Becher et al.

of the Cu(II) ion. In this method, a polydentate ligand where the thiols are protected as t-butyl mercaptides, reacts with a Cu(II) salt at an elevated temperature. The Cu(II) ion cleaves the S-Bu^t linkage and binds to the free thiolates thus formed (Fig. 16). This method is not only easy but also gives the product with a high yield. Also, as the thiol group is protected, multidentate ligand design becomes much easier. In their first report [79] showing the utility of this method, Toftlund and coworkers have isolated a number of cis-CuN_2S_2 complexes in the solid state (Fig. 17). Once formed, these complexes were found to be air-stable as in the case of the CuN_2S_2 complex with the ethylene-bridged cysteine dimer [73]. However, structural data are not available on these complexes apparently because none of these complexes could be obtained in single crystal form suitable for X-ray crystallography.

In order to model the blue site, the following sterically constrained ligand was used [80] that enforced a pseudotetrahedral N_2S_2 coordination (Fig. 18). The dihedral angle between the CuS_2 and the CuN_2 planes is 51.9° compared to 90° required for a strictly tetrahedral geometry. The Cu(II)-thiolate bond distance (2.245 Å) in this complex compares well with other cis-CuN_2S_2 complexes. Using this methodology, two ligands (Fig. 19) bearing N_2S_2 donar set have been designed [81] so that the two nitrogens have a large bite angle at copper forcing the geometry from planarity towards tetrahedral. This method has also been utilized [82] to synthesize a Cu(II) complex (Fig. 20) in the solid state with the donor set N_2S^*S (S* = thioether), the same as the one present at the plastocyanin active site. Unfortunately, no structural data could be reported for these complexes as they could not be obtained in single crystal form.

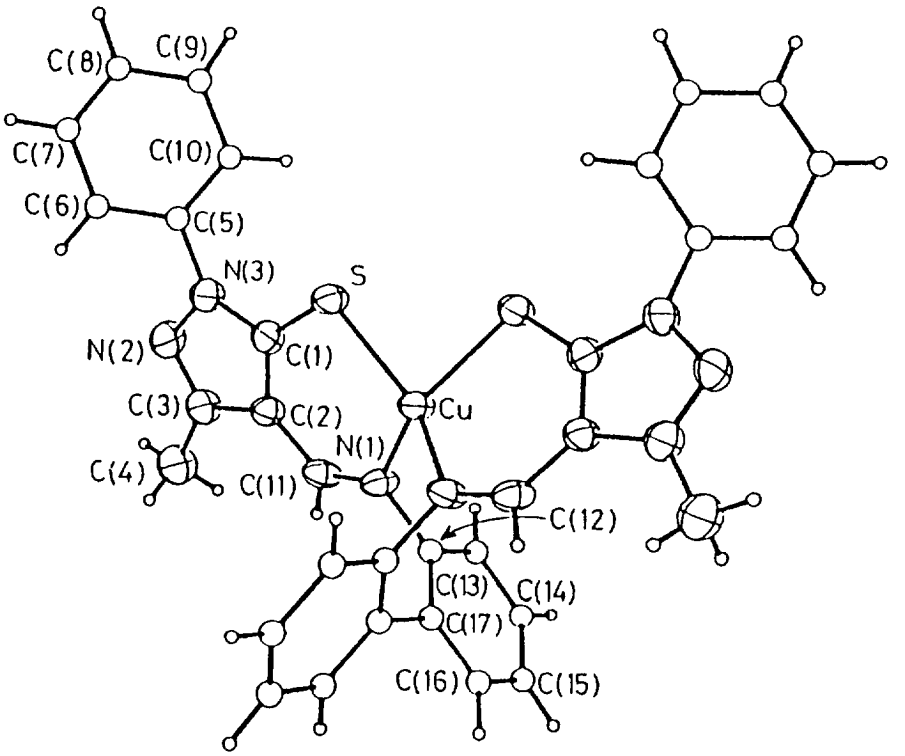


Fig. 18. The structure of pseudotetrahedral CuN_2S_2.

A large number of hexadentate Cu(II) thiolates with the donor set, $N_2S_2^*S_2$ (S* = thioether) has been synthesized [83,84] using t-butyl protected thiols (Fig. 21). In these complexes the spectroscopic data point to the tetragonal geometry around the metal ion with the four sulfurs in the equatorial plane (Fig. 22). This arrangement of donor atoms not only lowers the Cu(II)/Cu(I) couple (*vide infra*), but also makes possible the generation of Cu(III)-thiolate species which are metastable. These Cu(II)-complexes appear to be the first set of examples of hexadentate Cu(II)-thiolates. Of course, with the macrocyclic complex, $[Cu(cyclam)]^{2+}$, pentafluorothiophenolate forms [85] a tetragonal CuN_4S_2 complex (Fig. 23). In this complex, however, the axially disposed thiolates are weakly bound (Cu(II)-S distance is 2.940 Å) and have no spectroscopic signatures characteristic of the Cu(II)-thiolate bond (*vide infra*).

Becher and coworkers adopted another new strategy to make Cu(II) thiolates. They synthesized [86] a Cu(II)-thiolate starting from a polydentate ligand containing two terminal pyridine thione groups. This thione group assumes the tautomeric pyridinium thiolate form when bound to the cupric ion (Fig. 24). The strong electron withdrawing substitution of mercaptides such as in B should make the ligand less oxidizable and, therefore, enhance the stability of the mercapto-copper(II) bond

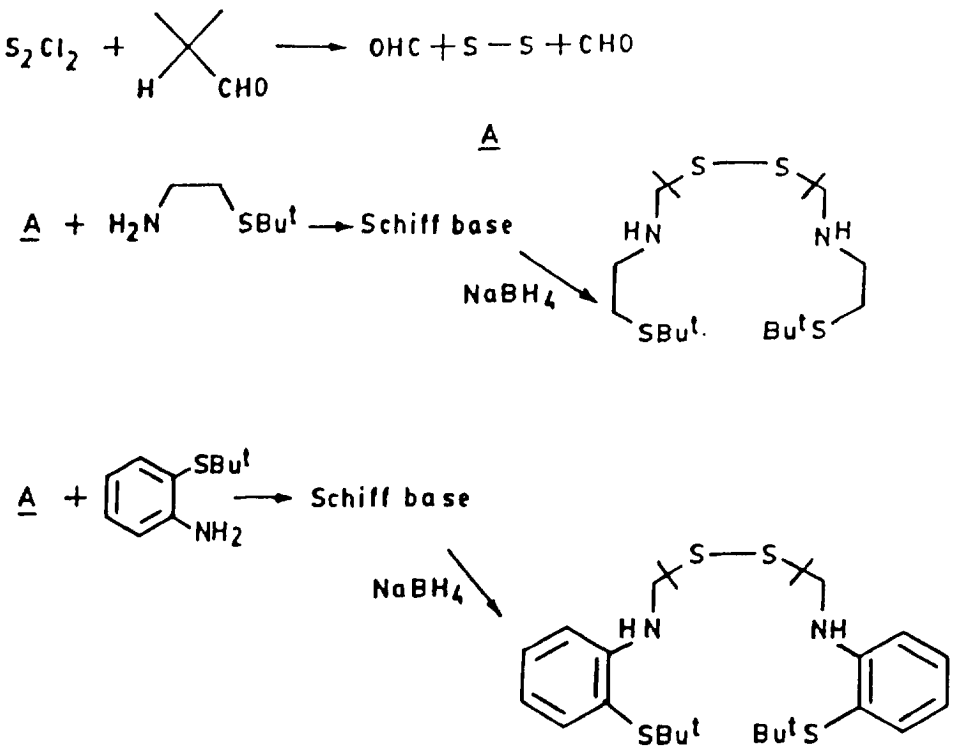
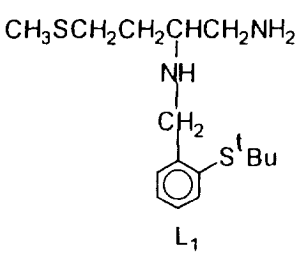


Fig. 19. The ligands that give a large bite angle at the metal ion.



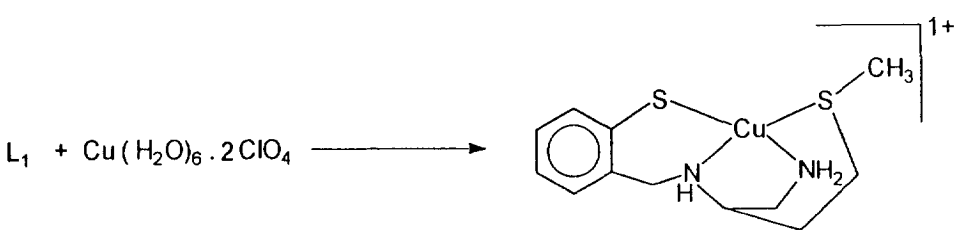


Fig. 20. The CuN_2S^*S (S* = thioether) complex and the ligand.

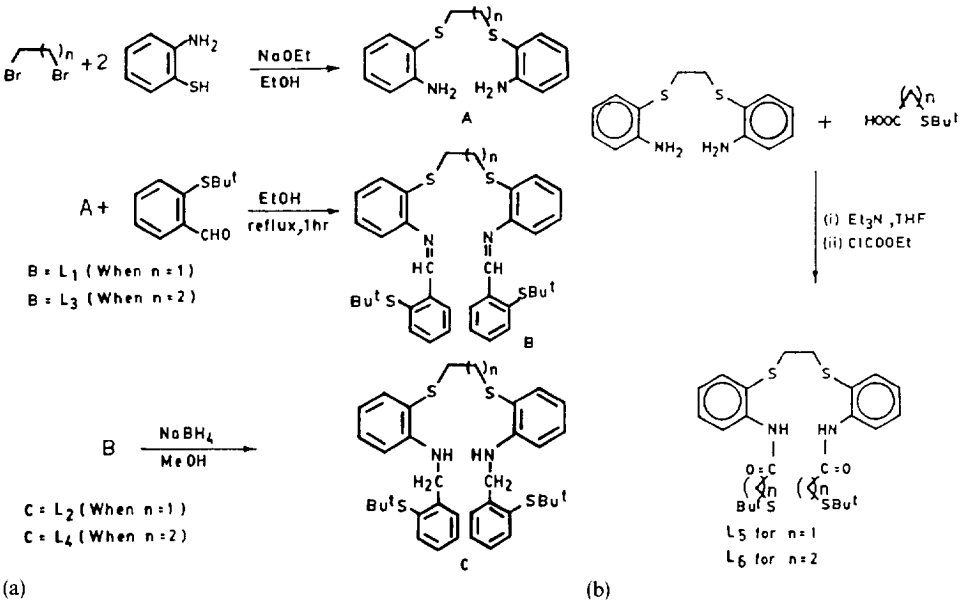
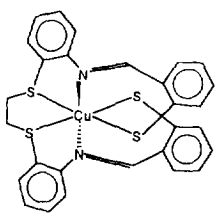


Fig. 21. a) Non-amide hexadentate $N_2S_2^*S_2$ (S* = thioether) donar ligands b) Amide hexadentate $N_2S_2^*S_2$ (S* = thioether) donar ligands c) Non-amide $CuN_2S_2^*S_2$ complexes d) Amide $CuN_2S_2^*S_2$ complexes.

compared to that of an aliphatic mercaptan. In addition, phenyl substitution at the pyridine nitrogen in (B) is expected to provide considerable steric hindrance to the generally easy coupling of copper(II) thiolate residues to produce disulfide units. Therefore, 1-phenyl-3-formyl-2(1H)-pyridinethione can provide an easily conjugable frame to form stable mercapto-copper(II) complexes. Casella and coworkers reported [87] the copper(II) complexes (Fig. 25) with imine ligands obtained from the condensation of 1-substituted 3-formyl-2-(1H)-pyridinethione and a series of 2-aminothia-alkyl-imidazole or benzimidazole. The same group reported [88] the following model system that gives N_2SS^* ligation at the metal centre (Fig. 26).

In order to find yet another new route to Cu(II)-thiolates, we proposed to try to cleave a S-S bond with the Cu(I) ion. Transition metal ions such as Fe(II), Ti(III) and Co(II) have been used [89–91] to reduce disulfide to the corresponding thiolates which then coordinate the metal ion forming metal thiolates. In such reactions, the formal oxidation state of the metal ion increases by one with electron transfer from the metal to the disulfide. Acyclic disulfides do not lead to the desired product. Therefore, we started with the idea that if a Cu(I) ion could be anchored in a macrocycle by bond formation with donors that include two nitrogens and a disulfide, then this might facilitate electron transfer from Cu(I) to the disulfide to reduce it to thiolates. Also, the resulting cis-N_2S_2 donor ligand will form a chelate with the metal ion in a square planar fashion. This method worked [92] well with both nine- and ten-membered macrocycles (Fig. 27). Dark red solids could be isolated from the reaction mixture. Although no structural conclusion could be made, it was



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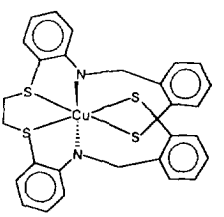

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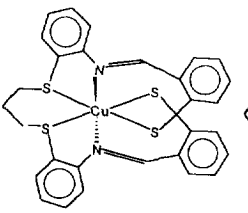

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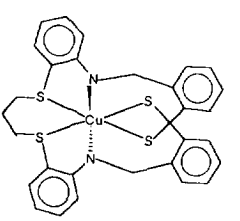

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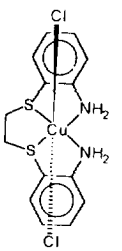

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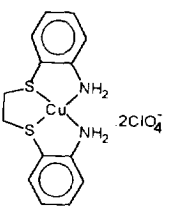

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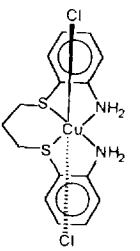

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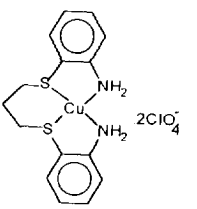

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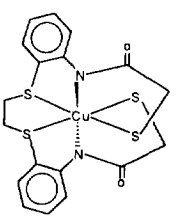

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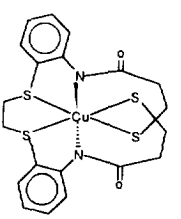

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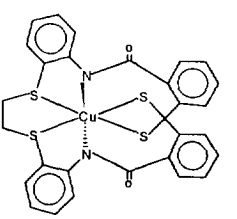

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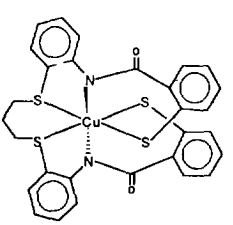

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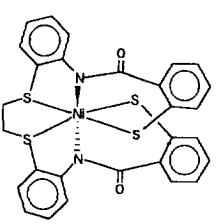

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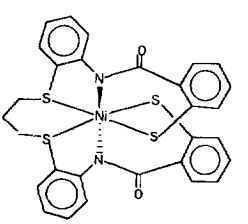

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shown through spectroscopic means that cis-CuN_2S_2 complexes had been formed (*vide infra*). This method also might afford pseudotetrahedral CuN_2S_2 complexes via ligand design which is not yet available.

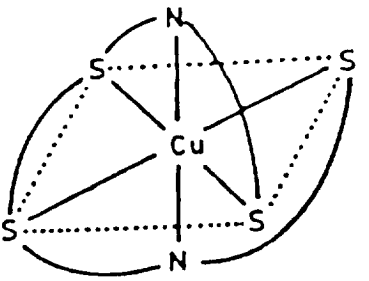


Fig. 22. The binding mode of the hexadentate ligands towards Cu(II).

2.3. *Physico chemical studies of copper(II)-thiolates*

In order to duplicate the spectroscopic features exhibited by the type 1 sites of blue proteins as well as other non-blue centres, the synthesized copper(II) thiolates have been subjected to spectroscopic and electrochemical techniques in addition to X-ray crystallographic characterization whenever possible. The spectroscopic tools mostly used are UV-vis and EPR spectroscopy. Only sporadically circular dichroism and resonance Raman spectroscopy have been used to aid in the elucidation of electronic structure and bonding. These studies are now discussed under separate headings.

2.3.1. *UV-Vis spectroscopy*

The peculiar ultraviolet, visible and near-infrared spectroscopic data obtained with the type 1 site has intrigued synthetic chemists for a long time. While there are variations among the type 1 sites from different proteins, the spectral features are characterized by a number of ligand field transitions in the near-infrared region and LMCT transitions in the visible as well as ultraviolet regions [5,93–102]. The spectral data on plastocyanin are collected in Table 5. The electronic transitions that led to such a complicated spectra have been identified using circular dichroism (CD) [100], magnetic circular dichroism (MCD) [101] and optical rotatory dispersion (ORD) [102] data. Electronic spectroscopic studies [103,104] on Co(II)- as well as Ni(II)-substituted enzymes have also substantiated these results. The dominant feature in the electronic spectra of these type 1 sites is the occurrence of an intense band (molar Cu absorptivity ~4000–6000) centred around 600 nm which makes these proteins deep blue in colour.

The electronic consequences, when thiols are converted to thiolates and coordinate Cu(II) in a type 1 geometry have been described by Solomon and coworkers [105]. To approximate the plastocyanin site, the C_S symmetry with the hypothetical molecular formula $[Cu(NH_3)_2(CH_3S)(CH_3SCH_3)]^+$ was used in their SCF-X_α calculations. These theoretical calculations in association with polarized spectroscopy on plastocyanin single crystals [106], low temperature conventional absorption [93] and MCD and CD spectral studies [105] on plastocyanin, azurin and stellacyanin have led to a clear picture of the thiolate ligation as present in the type 1 site and the charge transfer transitions associated with it. Band 4 (Fig. 28) which is the most intense

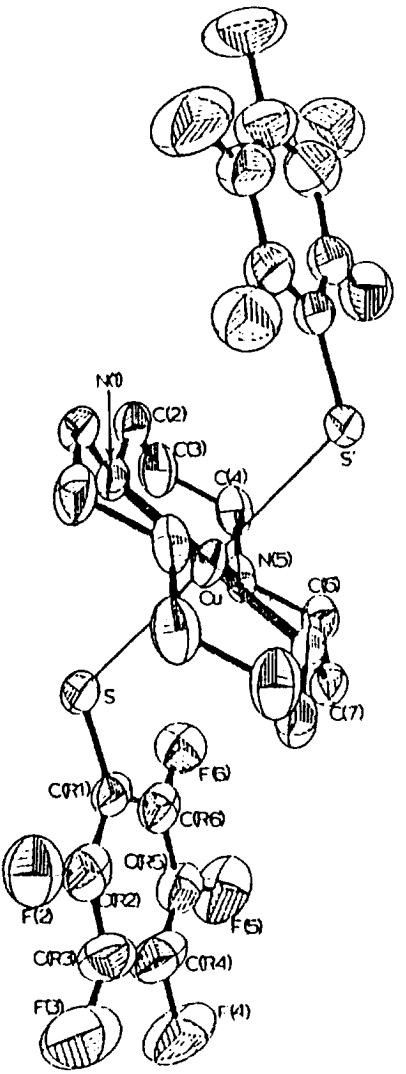


Fig. 23. The structure of [Cu(cyclam) $(SC_6F_5)_2$].

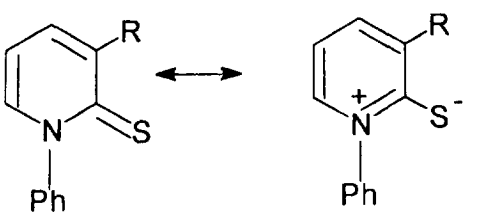
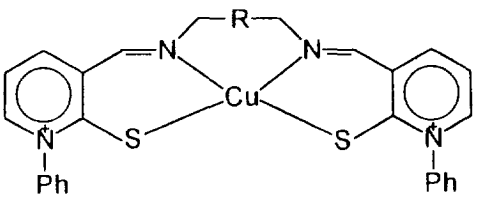
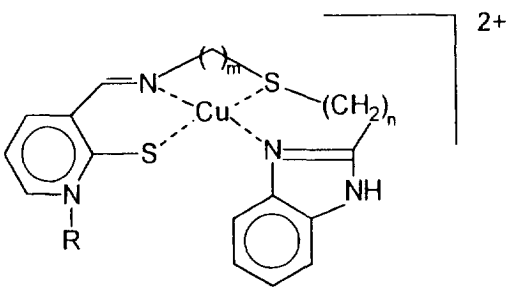


Fig. 24. The tautomeric forms of the pyridinethione ligand.



$R = CH_2CH_2$, $CH_2CH_2CH_2$, $CH_2CH_2CH_2CH_2$

Fig. 25. The Cu(II) complexes with the pyridinethione ligands.



	R	m	n
1a	Ph	2	2
1b	Ph	2	1
1c	Ph	3	1
1d	Ph	4	1
1e	i-Pr	2	1
1f	i-Pr	3	1
1g	i-pr	3	2
1h	i-Pr	4	1

Fig. 26. The CuN_2S*S complex of the pyridinethione ligand.

band in the spectrum is assigned as a $S(p\pi) \rightarrow Cu(d_{x^2-y^2})$ LMCT transition. Flanking on either side, two weak bands of σ-character appear. The lower energy band may be associated with a ligand field absorption. The high intensity of the $S(p\pi) \rightarrow d_{x^2-y^2}$ CT transition derives from extremely good overlap between the ground state $d_{x^2-y^2}$ orbital with the $S(p\pi)$ orbital of the thiolate in the C_S symmetry. Initially this band was assigned as a $S(p\sigma) \rightarrow d_{x^2-y^2}$ transition [105]a. As this picture unfolds, it is now clear that thiolate LMCT involves excitation from three occupied MOs having mixed S-Cu character into an acceptor orbital similarly constituted.

Initial attempts at duplicating the electronic spectral signature of the blue proteins involve solution studies of metastable Cu(II)-thiolate species. One of the early reports of electronic spectral studies [69–71] of Cu(II)-thiolates involved Cu(II) complexes with sulfhydryl containing peptides. These metastable species in solution

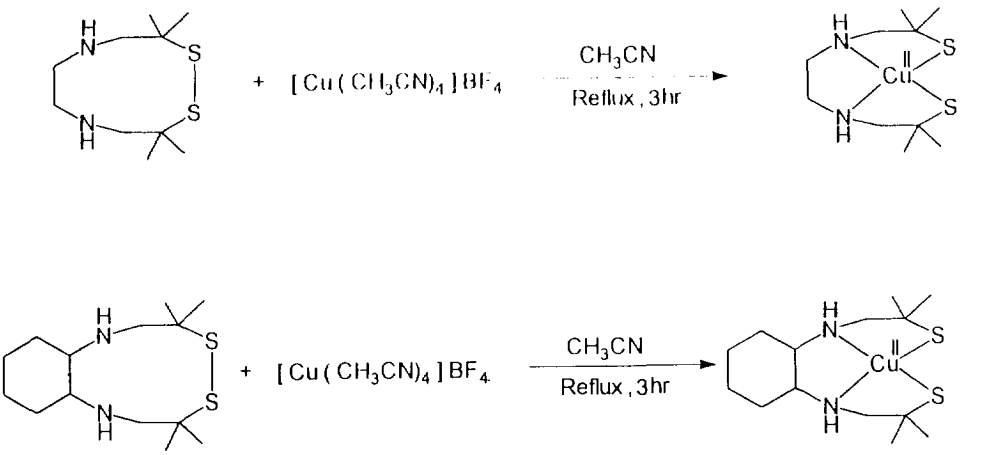


Fig. 27. Synthesis of cis-CuN_2S_2 complexes by the cleavage of S–S bonding.

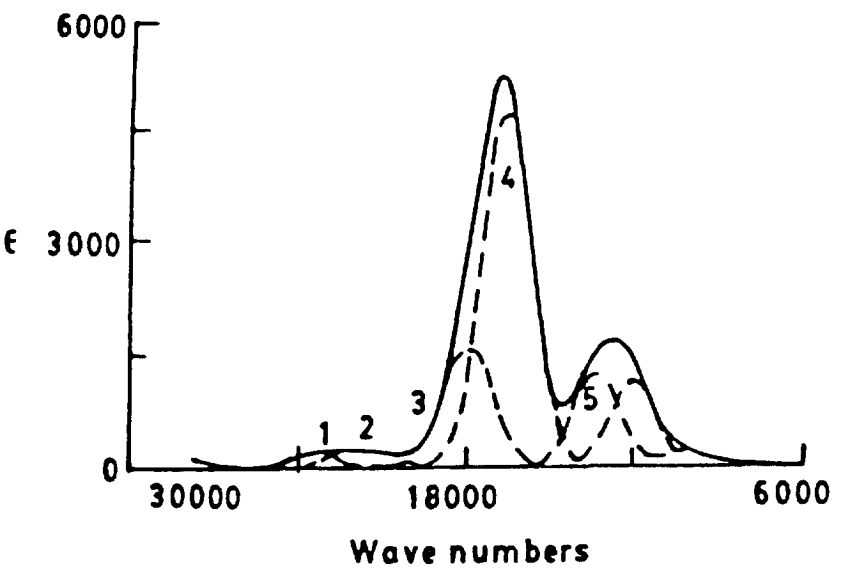


Fig. 28. The low temperature absorption spectrum of plastocyanin. The dashed lines indicate gaussian deconvolution of the spectrum.

exhibit a number of absorption bands in the visible region. To facilitate assignment of the spectra, they have reported both the CD and MCD data. The band that occurs around 600 nm with an extinction coefficient higher than 100 has been assigned as involving thiolate→Cu(II) LMCT transition. However in these studies, no conclusive evidence is provided for either the presence of Cu(II)-thiolate bonding or the existence of a single complex species in solution. The solution species can often be a complex mixture and may not lead to a definitive picture of the electronic

structure of Cu(II)-thiolate bonding. The effect of coordination geometry on the location of the thiolate→Cu(II) CT absorption has essentially been ignored in these studies. For tetragonal complexes, the CT bands involving thiolate and Cu(II) should occur in the UV or near-UV region (vide infra). Note that Cu(II) complexes with non-reducing ligands can give [107] intense ligand field transitions ($\varepsilon \sim 1000$) in the visible region. Moreover, instability in solution of Cu(II)-thiolate species can often lead to erroneous interpretation of spectral data. Bosnich and coworkers have provided [61] a clearer picture of the electronic spectroscopic characteristics of Cu(II)-thiolates. They have studied an adduct of $[Cu(cyclam)]^{2+}$ with isopropyl mercaptan in solution. This presumably square pyramidal CuN_4S chromophore shows a weak band near 360 nm ($\varepsilon \sim 200$) which is absent in the parent $[Cu(cyclam)](ClO_4)_2$ complex. This band has been assigned as an LMCT absorption involving the mercaptide and Cu(II). Poor overlap of the axially disposed thiolate with the $d_{x^2-y^2}$ orbital of Cu(II) is an explanation for the low intensity of this transition.

Electronic spectroscopic studies on trigonally distorted CuN_3S species formed by reacting trispyrazolylborate complexes of Cu(II) with different thiolates have given interesting results. With p-nitrothiophenolate [66], a broad absorption band appears around 600 nm with extinction coefficient in the range, 2000–4000 $M^{-1}\,cm^{-1}$ which has been assigned as due to σ(thiolate)→Cu(II) LMCT transition. The assignment has been reinforced by comparing the spectra using different thiols as well as those of corresponding Co(II) complexes and invoking the optical electronegativity principles of Jorgensen [107]. Another group has used [67] a hindered tris-pyrazolyl borate to synthesize a tetrahedral complex of Cu(II) with t-BuSH with the chromophore, CuN_3S. The tetrahedral structure is preserved in a non-coordinating solvent like CH_2Cl_2 and shows an intense band at 608 nm ($\varepsilon > 3500$ at 257 K) attributable to σ(thiolate)→Cu(II) LMCT absorption. The ligand field transitions for this complex appear around 900 nm. In a recent report [68] by this group, the X-ray crystallographically characterized trigonal pyramidal $[Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3]$ has been subjected to electronic spectral measurements. The complex exhibits a very strong band at 625 nm, with additional bands at 440 and 918 nm. One more band at 357 nm is reported which they suggest could be due to a decomposed product. As no solid state spectrum is available, the integrity of the complex in solution cannot be commented upon.

Early definitive conclusions regarding the electronic spectral characteristics of Cu(II)-thiolate species have been provided by Schugar's group. In the case of trigonal bipyramidal CuN_4S complex [58] $[Cu(tet\text{-}b)(o\text{-}SC_6H_4CO_2)].H_2O$, the nature of the electronic spectrum in solution is the same as the solid, but the intensities of the solid state spectrum are about twice as large as those obtained from solution. The band positions at 920, 730 and 590 nm have been assigned as ligand field absorptions. Absorption bands at 360, 418 and 430 nm are assigned to S→Cu(II) LMCT. The existence of a single d vacancy on Cu(II) coupled with the presence of three different lone pairs on the mercaptide sulfur can in principle result [105] in three LMCT absorptions with the most intense one appearing in the middle. The relatively intense high-energy absorption at 360 nm had been assigned as σ(S)→Cu(II) LMCT. The red shifted and weaker absorptions at 418 and 430 nm are assigned to π(S)→Cu(II) LMCT. The possibility of N→Cu(II) charge transfer

transition is ruled out as they appear [108] at energies above 300 nm. The occurrence of two weaker absorptions at lower energy may be due to the interaction of sulfur orbitals with the π-system of the benzene ring [109]. The separation in energy between the σ- and mean π-symmetry thiolate LMCT transitions in the complex ($4500\ cm^{-1}$) compares well with the range of $3800–4800\ cm^{-1}$ reported [93,98] for possibly analogous absorptions in the case of some type 1 sites. The blue shifts in the band positions are expected as the coordination geometry is very different. Moreover, the tetramine macrocycle being a strong donor is expected to destabilize the Cu(I) state when compared to the imidazoles in the native enzymes. Thus, the ligand field strength should be much higher in the case of model complexes. The corresponding bands in the structurally similar aliphatic analogue [Cu(tet)$SCH_2CH_2CO_2 \cdot 2CH_3OH$] [63] are similarly assigned. The hydrosulfide complex [64] has similar spectral features but is more complicated [110] because of the hydrosulfide group rather than a simple thiolate attached to Cu(II) ion. The binuclear copper(II) complex, $Cu_2(cyclops)_2(SC_6H_4\text{-Me-p})][ClO_4]$ [60] exhibits a strong absorption maximum at 415 nm with a shoulder around 360 nm in dichloromethane. Both these bands are attributed to sulphur to copper charge transfer transitions in the dimer unit. A few more such well-characterized dimers have to be studied by UV-vis spectroscopy before any unambiguous conclusions can be drawn about the CT transitions.

A clearer picture on the electronic spectroscopic characteristics of Cu(II)-thiolate bonding emerged from studies with four coordinate cis-CuN_2S_2 complexes. The UV-vis spectra of the complexes show a weak d-d band in the region 800–830 nm ($\varepsilon \approx 100$), the position of which is dependent upon the bite angle provided by the two amino N atoms. This variation fits the expected variation of a $^2A_1 \rightarrow {}^2B_2$ (D_{2d}) transition in a CuL_4 chromophore, distorted monotonically from planar towards a tetrahedral geometry [111–115]. The X-ray crystallographically characterized pseudoplanar CuN_2S_2 complex derived from an ethylene-bridged cysteine dimer also exhibits [73] a strong intensity band at 360 nm attributable to a σ(thiolate)$\rightarrow$Cu(II) LMCT transition. The other two bands involving Cu(II) and thiolate are not seen and could be mixed with other transitions. Most importantly, the integrity of the solid state structure remains intact in solution. Also, the mixed valent $Cu(II)_2Cu(I)_5$-complex [74] with the same ligand and two cis-planar CuN_2S_2 units (Fig. 15) shows a strong band around 360 nm attributable to the σ(thiolate)$\rightarrow$Cu(II) LMCT transition. The UV-vis spectra for the two mixed valent clusters [76,79] are too complicated to provide any useful insight. The pseudotetrahedral complex [80] with a twist angle of 51.9° (for an ideal tetrahedral geometry this angle will be 90°) show two intense transitions centred at 535 ($\varepsilon = 3000$) and 656 nm ($\epsilon = 1500\ M^{-1}\ cm^{-1}$). The LMCT nature of the band at 535 nm is shown by the observation of strongly resonance enhanced Cu-S vibrations (around $300\ cm^{-1}$) in resonance Raman experiments. As the S(σ)$\rightarrow$Cu(II) transition is expected to be strongly coupled to the Cu-S vibrations and the S(π)$\rightarrow$Cu(II) is expected to be weakly coupled, the transition at 535 nm can be assigned as the σ component and the 656 nm transition as the π component. Pseudotetrahedral CuN_2S_2 complexes [81] shown in Fig. 19 do not show solvent coordination to a noticeable extent. These complexes also preserve their solid state structures in solutions. Ligand field bands

appear at 815 and 760 nm red-shifted to a great extent compared with planar CuN_2S_2 chromophores [73,79]. The coordination geometry around copper in these two complexes is supposed to be very distorted from planarity. The σ(thiolate)→Cu(II) LMCT transitions appears near 350 nm for both complexes and the lower energy π(thiolate)→Cu(II) absorption appears near 470 nm. The high energy π-symmetry absorption is not seen in either complex. Compared to these cases, the tetragonal $CuNS_2^*S$ complex [82] shows a broad band at 760 nm due to d-d transition while the charge transfer transition involving thiolate occurs around 345 nm ($\varepsilon \sim 5470$). Becher et al. followed by Casella and coworkers have reported [86–88] electronic spectral investigations on their Cu(II)-thiolato complexes based on pyridine thione ligands. Together with intraligand transitions, identified by examining the spectra of Zn(II) and Cu(I) complexes of the ligands, the near-UV and visible regions contain a number of moderately intense bands in the Cu(II)-complexes. The strong band in the region, 410–435 nm ($\varepsilon > 3500$) in the Cu(II)-complexes originates from the enethiolate sulfur donor and can be attributed to the $\sigma(S^- \rightarrow Cu(II))$ LMCT transition. The lower energy $\pi(S^- \rightarrow Cu(II))$ LMCT absorption located in the region, 510–535 nm ($\varepsilon > 1500$) partially overlaps with the ligand field bands occurring in the range, 610–650 nm. However, no solid state spectra have been reported by this group. The intensity of the bands decrease rapidly at low temperatures signifying instability of the complexes in the medium. Thus, the extinction coefficient reported may not be as accurate as claimed by the authors.

Our hexacoordinated [83,84] copper(II) thiolato complexes ($CuN_2S_2^*S_2$, where S* = thioether) provide the first set of examples of hexacoordinated Cu(II) complexes with thiolate ligation. All the hexadentate complexes in this series show an intense band centred around 600 nm ($\varepsilon \sim 4500$ or higher) which make these complexes dark blue in color. Electronic spectral studies on a number of structurally characterized tetragonal complexes with $CuS_2^*N_2X_2$ (X = O, Cl; S* = thioether) and $CuN_4S_2^*$ chromophores have been reported [116,117] where the σ(N)→Cu(II) transition occurs around 280 nm while (S*)→Cu(II) transition could be located in the region, 340–540 nm. The tetradentate precursors (Fig. 21C) which do not have Cu(II)-thiolate ligation, show only a very weak ($\varepsilon \sim 100\ M^{-1}\ cm^{-1}$) absorption. Thus, the 600 nm band observed in the hexavalent $CuN_2S_2^*S_2$ complexes can be assigned as the σ(thiolate)→Cu(II) transition. The high intensity of this band is due to good overlap between the equatorially bound thiolate and the $d_{x^2-y^2}$ orbital on Cu(II) (Fig. 22). The shoulder that appears between 500–540 nm should then be the higher energy π(thiolate)→Cu(II) absorption. The lower energy σ-symmetry σ(thiolate)→Cu(II) transition may be mixed with the shoulder at 730–750 nm. The low-energy observed for the σ(thiolate)→Cu(II) transition for tetragonal coordination geometry is truly remarkable. X-ray structural studies of these or similar complexes are necessary to get a clear picture of structure and bonding in such complexes.

The X-ray structure of the hexavalent complex [Cu(cyclam)$(SC_6F_5)_2$] [85] is known where the two thiolate S atoms are not strongly bonded to the metal ion. This bond is found to be somewhat non-covalent in character and the electronic spectral characteristics of the complex is similar to the precursor complex, [Cu(cyclam)]$(ClO_4)_2$.

Table 5
EPR spectral data for the copper proteins

	$g_\perp$		$g_\parallel$		$A_\perp$		$A_\parallel$	Ref.
	g_x	g_y	g_z	A_x		A_y	A_z	
Plastocynin	2.042	2.059	2.226	–		–	63	[1–4]
Azurins								
Alcaigenes denitrificans		2.059	2.225	–		–	60	[1–4]
Pseudomonas aeruginosa		2.052	2.260	–		–	60	[1–4]
Pseudomonas denitrificans		2.055	2.260	–		–	60	[1–4]
Stellacyanin	2.025	2.077	2.287	54		28	37	[1–4]
Amicyanin		2.046	2.239		36		59	[1–4]
Auracyanin	2.018	2.062	2.210	62		12	47	[1–4]
Cucubber basic blue	2.02	2.08	2.207	60		10	55	[1–4]
Mavicyanin	2.025	2.077	2.287	54		28	37	[1–4]
Rustcynin	2.019	2.064	2.229	61		19	47	[1–4]
Umecyanin		2.05	2.317	–		–	38	[1–4]
Laccase Fungal:								
Polyspora versicolor		2.03	2.19	–		–	90	[1–4]
Laccase Tree:								
Rhus vernicifera		2.05	2.23	–		–	43	[1–4]
Ascorbate oxidase	2.058	2.036	2.227	–		–	58	[1–4]
Ceruloplasmin		2.06	2.215	–		–	92	[1–4]

2.3.2. EPR spectroscopy

EPR spectroscopy has been a very useful tool to understand the active sites of copper proteins. The most important EPR characteristics of the type 1 site which has intrigued chemists over the years is the small hyperfine splitting in the parallel region (Table 5). EPR studies of this site have revealed subtle yet important differences [118] between blue proteins such as plastocyanin, azurin etc. on the one hand and stellacyanin, a basic blue protein from cucumber (CBP) on the other. The plastocyanin class of blue proteins shows an essentially axial EPR spectrum. Only a small rhombic splitting ($g_x - g_y = 0.017$) could be detected [119] in the Q-band spectrum of poplar plastocyanin. The stellacyanin class of proteins shows [120] significant rhombic splitting ($g_x - g_y = 0.057$ for stellacyanin). The ground electronic states should, therefore, be different for the two classes of proteins. In probing the epr spectral characteristics of poplar plastocyanin, arguments [118] favour an anisotropic delocalization of the unpaired d-electron over the cysteine sulfur pπ orbital. However, the extent of the g splitting in stellacyanin cannot be accounted for by considering the excited state delocalization mechanism alone due to its small effects. It can be satisfactorily explained by taking into consideration about 3% mixing of the d_z2 orbital into the ground state wavefunction [118]. This amount of mixing can also satisfactorily explain the observed differences between A_x and A_y in stellacyanin. It remains to be seen whether the differences in the ground electronic states in the two groups of blue proteins can suggest their different biochemical reactivities. The wide range of protein g values (2.19–2.31) is greater than has yet been observed for low molecular weight

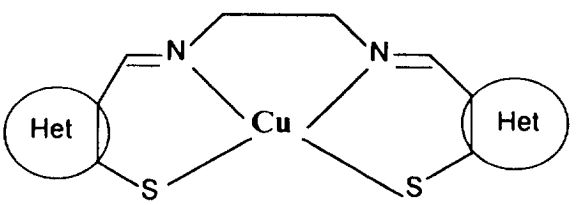
complexes. It appears that no variation solely in ligand geometry can result in such a range and that appreciable differences in the ligand identities must exist among the blue proteins. It also follows that the small hyperfine coupling in the parallel region can be achieved without having a thioether ligation [67].

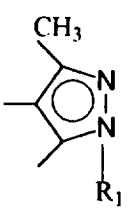
Serious efforts have been made by various workers to make Cu(II)-thiolates with small A_{II} values to model the type 1 site. Four coordinate Cu(II) complexes with one or more thiolate ligation were synthesized to duplicate the EPR characteristics of the blue site. The factors which determine [121] the magnitude of g in four coordinate cupric complexes are, in decreasing order of importance, the ligands, the coordination geometry and the overall charge of the complex. With respect to the ligand, Peisach and Blumberg [122] as well as Addison and Yokoi [123] have noted that g generally decreases along the series $O_4 > N_2O_2 > N_3O > N_4 > N_2S_2 > S_4$. This trend mirrors [124] an increasing nephelauxetic effect, increasing covalency, and decreasing ligand electronegativity, in accord with theoretical calculations [125]. For a given donor set, g_{II} increases as the coordination geometry is distorted from square planar towards tetrahedral. The overall charge on a cupric complex has a relatively small effect on g_{II}. However, Peisach and Blumberg had shown [122] that increasing the positive charge generally increases g_{II}. Generally, substitution of an N or O donor ligand for an S donor decreases the magnitude of A_{II} provided the coordination geometry as well as charge remain unaltered.

The four coordinate complexes of Cu(II) with sulfhydryl containing peptides described [69] by Sugiura and coworkers show only tetragonal EPR (Table 6) with the A_{II} values much higher than that observed in case of the blue sites. The perpendicular region shows prominent splitting. This splitting has been attributed to superhyperfine interactions with neighbouring nitrogen nuclei. Thus a square planar CuN_3S chromophore [70] has been proposed where all three N atoms are magnetically equivalent. Another green colored square planar complex [71] is formed by reacting α-mercaptopropionylglycine with cupric chloride. This complex contains a thiolate, a neighbouring deprotonated amide nitrogen and a terminal carboxylate group in addition to a water molecule in the coordination sphere. This $CuNO_2S$ complex exhibits $g_{II} = 2.259$, $g_{\perp} = 2.040$, and $A_{II} \sim 79 \times 10^{-4}$ cm^{-1} in solution at 77 K. The small A_{II} value has been attributed to the high covalency of the Cu-S bonding. However, for a square planar $CuNO_2S$ coordination, the significant rhombic distortion observed in the perpendicular region has not been satisfactorily accounted for. For complexes with the chromophore cis-CuN_2S_2 the EPR spectral characteristics depend quite strongly on the coordination geometry. In Table 6, the EPR parameters of the structurally characterized CuN_2S_2 complexes are collected. As the data indicate, with increasing twist angle the g_{II} value increases while the A_{II} value decreases as expected [122,123]. The g_{II} and A_{II} values do not change much in case of the tetragonal complex [82] with the chromophore CuN_2SS^* (same as in plastocyanin) underlying the importance of the coordination geometry in decreasing the A_{II} value. The ligands [81] shown in Fig. 19 are expected to enforce a pseudotetrahedral coordination geometry onto Cu(II). Indeed, the electronic absorption spectral characteristics (*vide supra*) point to a geometry substantially distorted from planar towards tetrahedral. Interestingly, the complex with the aliphatic ligand exhibits an EPR spectrum which is significantly rhombic-distorted (Fig. 29) and provides the

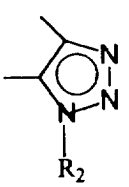
Table 6
EPR spectral data for tetradentate Cu(II)-thiolates

Complex	$g_{\parallel}$	$g_{\perp}$	g_o	$A_{\parallel}$	Ref.
CuN_3S (MAGH)	2.206	2.079	2.099	195	[70]a
CuN_3S (GGH)	2.177	2.065	2.109	206	[70]a
CuN_3S (MAGGG)	2.155	2.046	2.092	199	[70]a
$CuNO_2S$ violet (-MPG)	2.185	2.037	–	168	[71]
$CuNO_2S$ green (-MPG)	2.259	2.040	–	82	[71]
CuN_2SS	2.23	2.02	–	–	[82]
CuN_2S_2 as in					
a	2.139	2.020	–	184	[79]b
b	2.139	2.020	–	176	[79]b
c	2.170	2.020	–	170	[79]b
d	2.158	2.019	–	179	[79]b
e	2.135	2.020	–	175	[79]b
f	2.130	2.015	–	181	[79]b
CuN_2S_2	2.126	2.039	–	182	[73]
CuN_2S_2	2.155	2.036	–	137	[80]

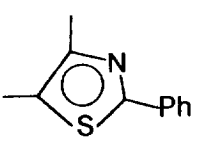




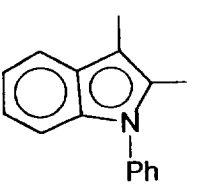
a CH_3 **b** Ph **c** p-NO_2-C_6H_4 $= R_1$



d CH_2-C_6H_5 $=R_2$



e



f

first example for such a spectrum with a Cu(II)-thiolate complex outside the stellacyanin class of proteins [118]. As pointed out above, in the case of stellacyanin the observed g split of 0.057 can be accounted for by considering mixing of about 3% of the d_{z^2} orbital into the ground state wavefunction. To evaluate the extent of d_{z^2}

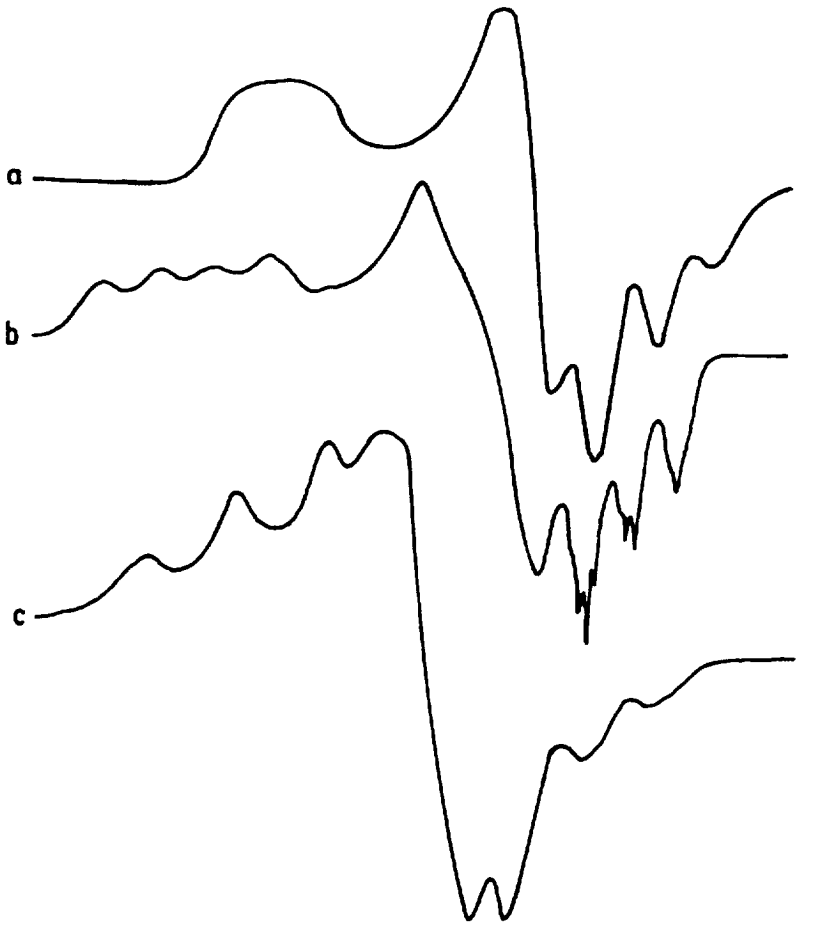


Fig. 29. The X-band EPR spectra of (a) stellacyanin (b) Cu(II)-doped [Zn (1,2-dimethylimidazole)$_2$Cl$_2$] and (c) CuN_2S_2 complex (in MeCN glass) of the aliphatic ligand shown in Fig. 19.

mixing neglecting the orbital reduction factors, a parameter R_g has been defined [126] as

$$R_g = \frac{2(g_x - g_y)}{g_x + g_y}$$

For stellacyanin, R_g is 0.9 while for the model complex above, this value is estimated to be about 1.6 which corresponds to about 4–5% mixing of the d_{z^2} orbital into the $d_{x^2-y^2}$ orbital. This amount of mixing also accounts for the observed difference in $(A_x - A_y)$ of 60×10^{-4} cm^{-1}. As a comparison, for the tetrahedral, X-ray crystallographically characterized Cu(II)-doped [Zn(1,2-dimethylimidazole)$_2$Cl$_2$] complex

Table 7
EPR spectral data for the CuN_3S complexes

Complex	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$	Ref.
$Cu\{HB(3,5\text{-}Me_2Pz)_2(SC_7H_7)\}(p\text{-}SC_6H_4NO_2)$	2.169	–	187	[66]
$Cu\{HB(3,5\text{-}Me_2Pz)_3\}(p\text{-}SC_6H_4NO_2)$	2.286	–	171	[66]
$Cu\{HB(3,5\text{-}Me_2Pz)_3\}(o\text{-}SC_6H_4NO_2)$	2.308	–	167	[67]
$Cu\{HB(3,5\text{-}iPr_2pz)_3\}(SBu^t)$	2.21	2.08	70	[68]
$Cu\{HB(3,5\text{-}iPr_2pz)_3\}(SCPh_3)$	2.23	2.07	71	[68]
$Cu\{HB(3,5\text{-}iPr_2pz)_3\}(SC_6F_5)$	2.30	2.10	52	[68]

[127] the value of R_g has been estimated to be about 1.1 which corresponds to about 3–4% mixing of the d_{z^2} orbital into the ground state.

Pseudotetrahedral CuN_3S complexes with tris-pyrazolylborate or substituted tris-pyrazolylborate and different thiolates show interesting EPR spectral results (Table 7). The complex $[Cu(HB(3,5\text{-}Me_2pz)_3\,(SC_5H_4\text{-}p\text{-}NO_2)]$ [66] exhibits an absorption spectrum (*vide supra*) reasonably similar to the type 1 centre but an EPR spectrum typical of a tetragonal copper due probably to ligation by a solvent molecule. That solvent can complicate the spectrum is further shown by studies with the hindered trispyrazolylborate ligand. The complexes, ($[Cu(HB(3,5\text{-}iPr_2pz)_3(SR)]$; SR = $tBuS^-$, Ph_3CS^- or $C_6F_5S^-$) show [67,68] EPR spectra quite similar to those found in the plastocyanin class of proteins in a non-coordinating solvent such as CH_2Cl_2. With solvents like DMF, both the UV-Vis and EPR spectra change greatly. Interestingly, the crystal structure of the complex with the thiolate Ph_3CS^- is [68] more like trigonal pyramidal rather than tetrahedral. In comparison, the active site geometry in plastocyanin has been described as flattened tetrahedron corresponding to the C_{3v} point group symmetry [105].

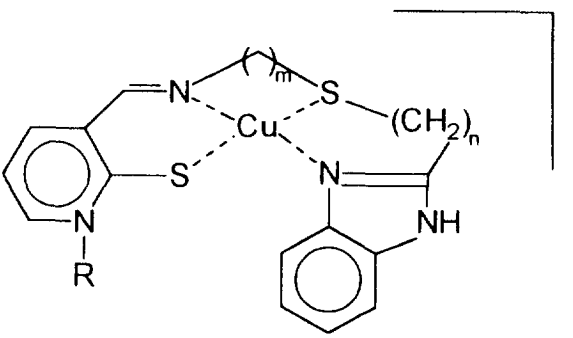
Four-coordinate Cu(II)-thiolato complexes [86–88] derived from pyridinethione ligands were also subjected to investigation by EPR. However, these systems are potentially complicated because of conjugation of the thiol group with the pyridine ring. These complexes show axial or nearly axial spectra with large A_{II} values (Table 8) in frozen solvent media. The EPR spectra of $[Cu(pyt\text{-}N,S\text{-}im)](ClO_4)_2$ and $[Cu(sal\text{-}N,S\text{-}im)](ClO_4)$ are similar and may actually be rhombic in character. However, the g value in the perpendicular region can only be obtained by simulation and the distortion from axial symmetry must be relatively small. The salient feature of the EPR spectra for the complexes is that as the bite angle between the NS-set of donors increases, the A_{II} value decrease. However, the lowest value of A_{II} obtained (Table 8) is still high compared to that of the type 1 site.

The EPR spectra of polycrystalline $[Cu(tetb)(SCH_2CH_2CO_2)].\,2CH_3OH$ are rhombic in appearance [63] with observed g values at 80 K of 2.047, 2.071 (poorly resolved) and 2.163 compared to the crystal spectrum (2.074, 2.086 (poorly resolved) and 2.117). The aromatic analogue [Cu(tetb)(o-mercaptobenzoate)] shows [58] a similar spectrum. These spectral results are appropriate for a CuN_4S chromophore with rhombic symmetry.

In the $[Cu(cyclam)(SC_6F_5)_2]$ complex [85], the relatively non-covalent nature of

Table 8
EPR spectral data for the Cu(II) complexes of pyridinethiones

Complex	$g_\perp$		$g_\parallel$	$A_\perp$		$A_\parallel$	Ref.
	g_x	g_y	g_z	A_x	A_y	A_z	
$Cu(Pyt_2\text{-en})$	2.020	2.020	2.140	31	31	184	[86]
$Cu(CH(=S)N(Me)O)_2$	2.033	2.034	2.15	35	40	190	[86]
$Cu(Pyt\text{-nbn})_2$	–	–	2.158	–	–	162	[87]a
$Cu(Pyt\text{-sbn})_2$	–	–	2.168	–	–	160	[87]a
$Cu(Pyt\text{-ptol})_2$	–	–	2.147	–	–	150	[87]a
$Cu(Pyt\text{-m-niph})_2$	–	–	2.151	–	–	145	[87]a
$Cu(Pyt_2\text{-1-2-diab})$	–	–	2.165	–	–	169	[87]a
$Cu(Pyt_2\text{-1,3-pn})$	–	–	2.149	–	–	176	[87]a
$Cu(Pyt_2\text{-1,4-pn})$	–	–	2.154	–	–	160	[87]a
$Cu(Pyt_2\text{-ornOMe})$	–	–	2.215	–	–	150	[87]a
1a	2.210		2.07	–	–	163	[87]b
1b	2.166		2.05	–	–	174	[87]b
1c	1.981		2.160	116		–	[87]b
1d	2.236		2.06	–	–	145	[87]b
1e	2.185		2.06	–	–	168	[87]b
1f	2.162		2.05	–	–	172	[87]b
1g	1.980		2.163	114		–	[87]b
1h	2.186		2.06	–	–	–	[87]b
Cu(Pyt-N,S-im)	2.04		2.163	–	–	176	[88]
Cu(Sal-N,S-im)	2.05		2.206	–	–	170	[88]
Cu(N,S-im)	2.055		2.287	–	–	176	[88]



	R	m	n
1a	Ph	2	2
1b	Ph	2	1
1c	Ph	3	1
1d	Ph	4	1
1e	i-Pr	2	1
1f	i-Pr	3	1
1g	i-pr	3	2
1h	i-Pr	4	1

the metal-sulfur linkages is suggested by the EPR data in DMF solution with excess thiolate present ($g_{II}=2.200$, $g_{\perp}=2.050$; $A_{II}=205\times10^{-4}\,cm^{-1}$, $A_{\perp}=30\times10^{-4}\,cm^{-1}$ at 77 K) and as a 5% dopant in the corresponding zinc compound ($g_{II}=2.166$, $g_{\perp}=2.039$, $A_{II}=205\times10^{-4}\,cm^{-1}$, $A_{\perp}=29\times10^{-4}\,cm^{-1}$, 300 K). The close similarity of these data with those of $[Cu(cyclam)](ClO_4)_2$ in DMF shows that the principle plane remains associated with CuN_4 core in the pure complex, its solution, and in the lattice of the Zn compound. The $A_{\perp}$ values in particular indicate similarly weak Z-axial interaction in all three complexes.

The hexacoordinated $CuN_2S_2^{\ddagger}S_2$ complexes [83,84] where the two thioethers and the two thiolates occupy the equatorial positions show an essentially axial EPR spectra in acetonitrile glass at 77 K. The spectra correspond to the ground state being $d_{x^2-y^2}$. The A_{II} values are significantly smaller than those obtained with tetragonal Cu(II) complexes (*vide supra*) and could be due to strong equatorial coordination by four sulfurs which can delocalize the single d electron on to available sulfur orbitals.

2.3.3. Electrochemistry

The high potential values for the Cu(II)/Cu(I) couple observed for the type 1 site (Table 9) has led to concerted efforts to model this site electrochemically. The high potentials observed for the blue sites are reflective of their geometry much distorted from the planar one favoured by Cu(II). This distorted structure is a compromise geometry acceptable to both the oxidation states so that their interconversion can be achieved easily. Interestingly, the redox potentials of the type 1 sites are a function of the pH of the medium. Below pH 7, the potential of the blue site in plastocyanin increases as the pH is decreased due to the protonation of His 87 at the active site.

Attempts have been made [128–130] to correlate the Cu(II)/Cu(I) potentials of model complexes with ligand topology. The Cu(II)/Cu(I) potential may be raised by any one of a combination of factors which lead to lowering LFSE: decreasing ligand σ-donor ability and constraining the geometry of the metal centre to the one

Table 9
Redox potentials (vs. NHE) for the blue copper proteins

	Redox potential, mV (pH)	Ref.
Plastocyanin	+370(7.0)	[1–4]
Azurins		
Alcaigenes denitrificans	+276(7.0)	[1–4]
Pseudomonas aeruginosa	+308(7.0)	[1–4]
Stellacyanin	+184(7.1)	[1–4]
Amicyanin	+261(7.0)	[1–4]
Auracyanin	+240(8.0)	[1–4]
Cucubber basic blue	+317(7.0)	[1–4]
Mavicyanin	+285(7.0)	[1–4]
Rustcynin	+680(2.0)	[1–4]
Umecyanin	+283 (7.0)	[1–4]

that favours Cu(I), e.g. tetrahedral. Presence of π-acceptor ligands also favour Cu(I). The flexibility of the ligand(s) could also be an important factor. The structural reorganisation accompanying a redox will affect the kinetics of the heterogeneous electron-transfer process. According to Marcus theory, inner-sphere rearrangements, enhancing the activation barrier of the electron transfer, slow down the rate of the process, so causing a departure from electrochemical reversibility [131]. With strong donors such as nitrogen and thiolate sulfur, a Cu(II) complex with the geometry distorted from planarity is expected to show high positive value for the Cu(II)/Cu(I) couple. Gray has elegantly pointed out that the lower the reorganisation following a redox change in copper complexes, the more positive is its thermodynamic redox potential [132].

Trigonal pyramidal Cu(II) complexes with $(HB(3,5\text{-}iPr_2pz)_3$ and different thiolates show striking similarities with the type 1 centres in their spectroscopic properties but their reduction potential values are significantly lower (Table 10). This is due to the strong donor ability [65] of the tris-pyrazolylborate group towards Cu(II). For the cis-CuN_2S_2 family of Cu(II) thiolates, only limited electrochemical data are available, often due to non-reversibility of the Cu(II)/Cu(I) couple in these complexes.

The pseudotetrahedral CuN_2S_2 complexes [81] shown in Fig. 19 give interesting electrochemical results (Table 10). Each exhibits a well-defined, quasi-reversible response in acetonitrile medium at room temperature with $E_{1/2} \approx 0.5$ V vs. SCE. This is the highest value obtained so far for a model CuN_2S_2 complex. Although X-ray structures are not available for these complexes, their UV-vis and EPR data suggest, in comparison with known structures, that the geometry in each case is close to tetrahedral.

The electrochemical results for the four-coordinate Cu(II) complexes [86–88] with pyridinethione ligands show a number of quasi-reversible peaks. One of them

Table 10
Redox potentials (vs. SCE) for tetradentate Cu(II)-thiolates

Complex	$E_{1/2}$, V Cu(II)/Cu(I)	ΔE_p, mV	Solvent	Ref.
$Cu\{HB(3,5\text{-}iPr_2Pz)_3\}(SBu^t)$	−0.11		DCM	[68]
$Cu\{HB(3,5\text{-}iPr_2Pz)_3\}(SCPh_3)$	−0.12		DCM	[68]
$Cu\{HB(3,5\text{-}iPr_2Pz)_3\}(SC_6F_5)$	0.26		DCM	[68]
$Cu(Pyt_2\text{-}en)$	−0.218	123	DMF	[86]
1a	+0.24	118	MeCN	[87]b
1b	+0.34	108	MeCN	[87]b
1e	+0.35	127	MeCN	[87]b
1f	+0.35	80	MeCN	[87]b
1g	+0.33	100	MeCN	[87]b
$Cu(Pyt_2\text{-}pn)$	+0.01	85	MeCN	[87]c
$Cu(Pyt_2\text{-}bn)$	+0.20	81	MeCN	[87]c
$Cu(Pyt_2\text{-}o\text{-}phn)$	+0.24	110	MeCN	[87]c
CuN_2S_2(aromatic)	+0.55	100	MeCN	[81]
CuN_2S_2(aliphatic)	+0.51	120	MeCN	[81]

For the complex numbers, refer to Table 8
DCM = Dichloromethane; DMF = Dimethylformamide.

is attributed to the Cu(II)/Cu(I) couple while the others are all ligand based redox processes. The Cu(II)/Cu(I) couple moves towards the positive side as the coordination geometry is distorted more and more towards tetrahedral (Table 10). However, conjugated systems usually give electrochemical data which are quite complex and couples may not be purely metal or ligand centred processes.

Pentacoordinated $CuN_4(p\text{-}ClC_6H_4S)$ and $CuN_4(C_6H_5S)$ (N_4 = cyclam) complexes [59] exhibit cyclic voltammetric patterns which are quasi-reversible, metal-centred responses. The $E_{1/2}$ values obtained for CuN_4-(p-Cl_6H_5S), $CuN_4(C_6H_5S)$ and $[CuN_4(OH)_2]$. BF_4 are −0.86, −0.85 and −0.49 V vs. SCE respectively, which indicate that the binding of the mercaptide ligands is associated with a stabilization of the copper(II) state by approximately 350 mV. This stabilization must arise from the shift to the highly distorted five-coordinated geometry of the above mentioned thiolato complexes, compared to $[CuN_4(OH)_2]$. BF_4. In addition, the greater donation of electron density to the metal by the more powerful mercaptide ligand contributes to the observed shift in potential by making the copper(II) more difficult to reduce. The dinuclear copper(II) thiolato complex, $[Cu_2(cyclops)_2(SC_6H_9Me\text{-}p)][ClO_4]$ [60] in dichloromethane containing 0.1 mol dm^{-3} of Bu_4N. BF_4 as a supporting electrolyte gave two quasi-reversible redox waves at $E_{1/2} = -0.416$ and at −0.679 V vs. NHE with E = 110 and 90 mV respectively, which may be assigned to two successive one electron processes (Eq. (1)) as confirmed from the EPR spectra under controlled potential electrolysis conditions at room temperature.

$$Cu(II)-S-Cu(II) \rightarrow Cu(I)-S-Cu(II) \rightarrow Cu(I)-S-Cu(I) \quad (1)$$

Electrochemical studies of tetragonal $CuN_2S_2^*S_2$ complexes [83,84] provided very interesting results which are collected in Table 11. In these complexes, the Cu(II)/Cu(I) potential is shifted to a very negative value and is not observable due to strong hexacoordination. Moreover, the strongly bound four sulfurs in the equatorial plane raise the energy of the singly occupied $3d_{x^2-y^2}$ orbital so that the

Table 11
Redox potentials (vs. SCE) for the CuN_2S_2 *S_2complexes in MeCN

Complex	$E_{1/2}$, V Cu(II)/Cu(I)	ΔE_p, mV	Ref.
1	0.53	110	[83]
2	0.55	180	[83]
3	0.51	430	[83]
4	0.55	450	[83]
6	0.50	60	[84]a
6^+	0.50	60	[84]a
7	0.54	160	[84]a
8	0.53	100	[84]b
9	0.48	120	[84]b

For the complex numbers, refer to Figs. 21c ad 21d

Cu(III)/Cu(II) potential decreases. Specifically, for the non-amide complexes (Fig. 21c), a well-defined cyclic response was obtained for the Cu(III)/Cu(II) couple with $E_{1/2}$ values in the range, 0.4 to 0.55 V vs. SCE (Table 11). An exhaustive constant potential coulometry performed on complex 1 at room temperature at 0.75 V gave $n=1.1$ e^-. However, the oxidized product was so unstable that it did not allow any spectral investigation. For the amide complexes (Fig. 21d), similarly

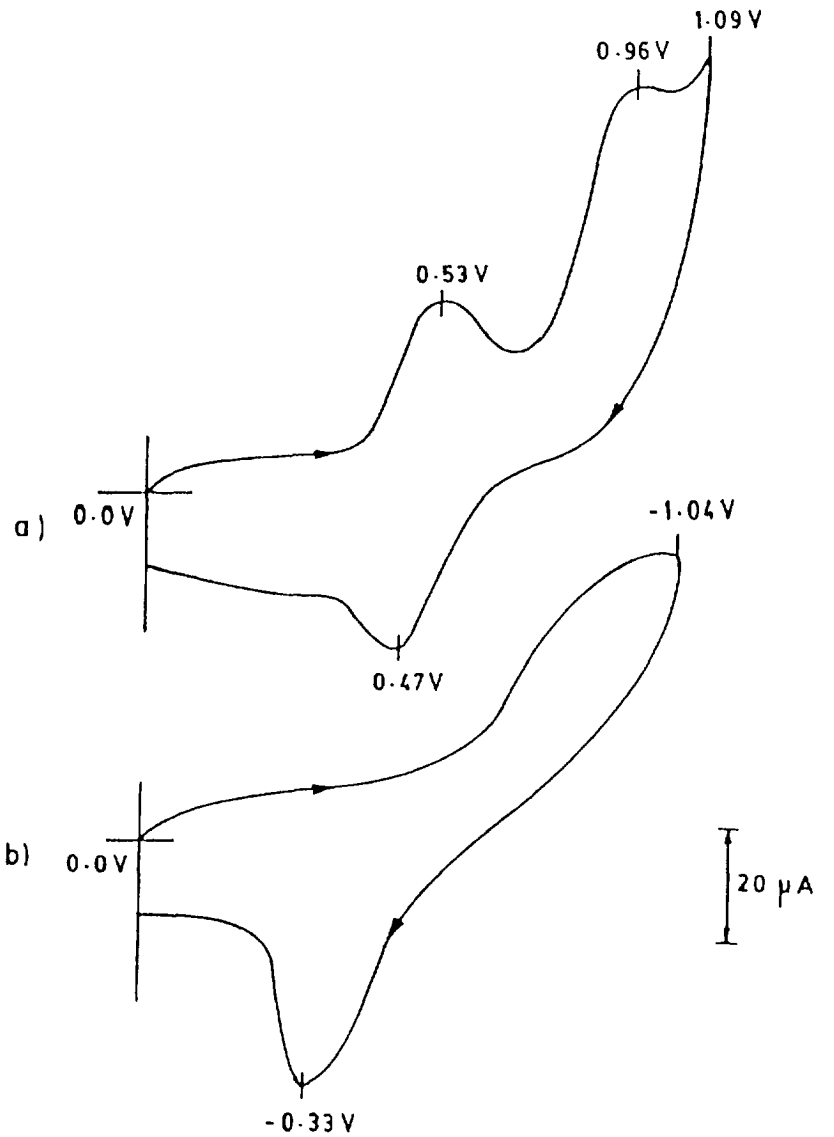


Fig. 30. Cyclic voltammograms in MeCN for the $CuN_2S_2^*S_2$ complex no. 6 shown in Fig. 21d (a) for positive scan (b) for negative scan at the rate of 50 mV s^{-1}.

the Cu(III)/Cu(II) potentials were observed around 0.5 V vs. SCE. Interestingly, complex 6 exhibited a reversible Cu(III)/Cu(II) signal (Fig. 30) with $E_{1/2} \approx 0.55$ V vs. SCE. On exhaustive controlled potential coulometry at 0.7 V at room temperature of an acetonitrile solution, the color of the solution changed from dark blue to dark green. The experimental value for n was found to be 1.02 e^- and the oxidation-reduction cycle could be completed with about 96% recovery of the starting complex. The oxidised product, did not show any EPR signal at room temperature or at liquid nitrogen temperature. However, the apparent diamagnetism could result from strong coupling of the unpaired electron localized on a Cu(II) centre and a free radical ligand, that is $[Cu(II)(L^{2-})] \rightarrow [Cu(II)(L^{\bullet -})]^+$ (a) $+e^-$ instead of $[Cu(III)(L^{2-}]$ (b) $+e^-$. In this case, (a) and (b) may be regarded as two limiting forms of a resonance hybrid system. The only question remained to be answered was which structure contribute more to the resonance hybrid. It is of relevance to mention here that Cu(III) has been implicated [133] in the biochemical reactions of some copper proteins.

3. Conclusion and future scope

A considerable number of copper proteins which contain thiolate ligation at the active site have been characterized by X-ray crystallography. However, spectroscopic and electrochemical studies on these metalloproteins are still required to understand their biochemical functions at the molecular level. The type 1 site due to its peculiar spectroscopic behaviour has attracted maximum attention from inorganic chemists. As mentioned at the outset, the type 1 active site present in the metalloproteins is intrinsic in nature and hence data obtained on them can be difficult to interpret. Surmounting the redox instability of Cu(II)-thiolate systems, a large number of complexes have been synthesized. However, they are not effective models for the blue site as they do not have tetrahedral coordination geometry. A systematic study of X-ray crystallographically characterized Cu(II)-thiolates is still required. However, it is not easy to have tetrahedral geometry around the Cu(II) ion with thiolate ligation due to its enhanced redox instability. Kitajima circumvented this problem using bulky trispyrasolylborate and mercaptans which elegantly answered some of the aspects of the type 1 site. To have an effective model for such an active site, the environment around the Cu(II) ion should be such that it is not easily attacked by reagents. Such an isolated system can be possible with macrocyclic and specially macropolycyclic cryptands having one or more mercaptans as pendant arms. Such systems can enforce a very distorted geometry on the metal ion and might take part in outer sphere electron transfer reactions in a reversible manner.

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