

## Tetranuclear Cu(I) Thiolate Complexes in Relation to the Metal Bonding Site of Yeast Copper–Thionein

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Attempts to mimic the tetranuclear  $\text{Cu}_4\text{S}_8(\text{Cys})$  center of yeast copper thionein with aliphatic mercaptanes reveal the  $(\text{CuSR})_n$  polymers to be strictly favoured over species with a coordination number higher than 2 for copper. However, using thiophenolates and tetraacetonecopper(I) perchlorate, crystalline tetranuclear  $[\text{Cu}_4(\text{SR})_6]^{2-}$  complexes were obtained as alkylammonium salts. Infrared spectra,  $^1\text{H}$  nuclear magnetic resonance, X-ray photoelectron spectrometry and analytical data support the suggestion of a pseudo-adamantane arrangement of the  $\text{Cu}_4\text{S}_6$  core, copper being purely Cu(I) coordinated exclusively to thiolate sulphur. As opposed to the proposed cubane-type structure of the  $\text{Cu}_4\text{S}_8(\text{Cys})$  center, i.e. four fused  $\text{CuS}_4$  tetrahedra, cuprous thiophenolate tetranuclear complexes are demonstrated to be restricted to three-fold coordination of the metal.

### Introduction

Yeast copper–thionein ( $M_r = 4800$ ) contains 4 Cu and 8 cysteine residues [1–3]. The sequence of amino acid residues in the protein is rather unusual. No aromatic residues are detectable; approximately 33% of the total residues are attributed to both glutamate and aspartate [2]. X-ray photoelectron spectrometry [2, 3] and indirect paramagnetic resonance measurements [4] revealed the exclusive presence of  $3d^{10}$  Cu. According to EXAFS data, each copper is tetrahedrally surrounded by 4 thiolate residues, two copper–sulphur distances being 2.22 Å, the other two 2.36 Å [3, 5]. For this tetrahedral coordination to be made up by a  $\text{Cu}_4(\text{SR})_8$  stoichiometry, a tetranuclear binding site is required. A cubane arrangement (Fig. 1), reminiscent of the  $(\text{Fe}_4\text{S}_4)$  cores [6] in sulphur proteins was proposed [3, 5].

Unfortunately, no X-ray diffraction data are available. Thus, for correlation of structural and spectroscopic data, we attempted to mimic the cubane

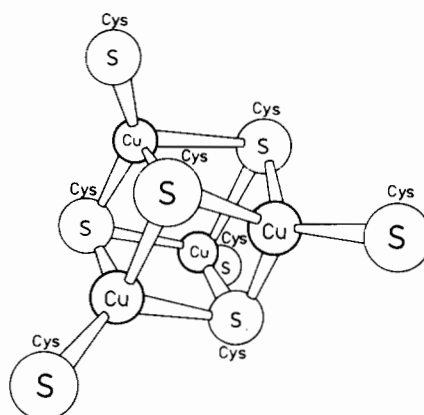


Fig. 1. Proposed cubane structure of the  $\text{Cu}_4\text{S}_8(\text{Cys})$  core of yeast copper–thionein [3, 5].

type structure in synthesizing a  $\text{Cu}_4\text{S}_8$  unit from tetraacetonecopper(I) perchlorate and thiolate compounds including both aliphatic and aromatic mercaptanes. Trimethoxysilylpropylmercaptane, N-2-mercaptopropionylglycine and thiourea were chosen as aliphatic, and thiophenol and *p*-fluorothiophenol as aromatic type sulphur compounds.

The prepared copper complexes were chemically analysed and characterized by ir,  $^1\text{H}$  nmr and X-ray photoelectron spectrometry. The latter method was useful to study the actual charge on both the copper and the coordinated sulphur.

### Experimental

All reagents were of analytical grade purity and commercially obtained.  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  was prepared as described by Hemmerich [7]. The reactions were carried out under dry nitrogen at 22 °C.

Infrared spectra were recorded on Beckman AccuLab 4 (4000–600  $\text{cm}^{-1}$ , KBr-discs) and Perkin-Elmer 597 ir spectrophotometer (600–200  $\text{cm}^{-1}$ , nujol mull), respectively.  $^1\text{H}$  NMR spectra were recorded using a Bruker WP 80 in  $d_6$ -acetone under dry nitrogen against trimethylsilane as internal standard. X-ray photoelectron spectroscopy was performed on a Leybold LHS-10 unit at  $-150^\circ\text{C}$  and  $10^{-7}$  mbar. The  $\text{C}_{1s}$  line at 284.0 eV obtained from the double adhesive cello tape (Scotch tape) served as an internal standard.

#### *N*-2-mercaptopropionylglycine Copper(I), 1

A solution of piperidinium-*N*-2-mercaptopropionylglycinate copper(I), 2 (2.14 mmol) in ethanol (40 ml) was acidified with hypophosphoric acid (5 mmol). *N*-2-mercaptopropionylglycine copper(I) precipitated as a white powder.  $(\text{C}_5\text{H}_8\text{CuNO}_3\text{S})_n$ , calc. C: 26.61, H: 3.57, N: 6.21; found C: 26.19, H: 4.03, N: 5.71.

#### Piperidinium-*N*-2-mercaptopropionylglycinate Copper(I), 2

To a solution of mercaptopropionylglycine (3.23 mmol) and  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (1.62 mmol) in acetone (25 ml) piperidine was added to yield white polymeric piperidinium-*N*-2-mercaptopropionylglycinate copper(I).  $(\text{C}_{10}\text{H}_{19}\text{CuN}_2\text{O}_3\text{S})_n$ , calc. C: 38.63, H: 6.16, N: 8.01, S: 10.31; found C: 38.82, H: 6.29, N: 8.50, S: 10.94.

#### 1-Mercapto-3-trimethoxysilylpropane Copper(I), 3

To a solution of trimethoxysilylpropylmercaptane (5.6 mmol) and piperidine (5.6 mmol) in acetone (20 ml),  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (1.53 mmol) in acetone (20 ml) was added slowly. The precipitate was filtered off, washed repeatedly with acetone and dried under vacuum.  $\text{C}_6\text{H}_{35}\text{CuO}_3\text{SSi})_n$ , calc. C: 27.84, H: 5.79; found C: 27.79, H: 5.62.

#### Benzenethiolato copper(I), 4

Thiophenol (4.86 mmol) and  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (0.83 mmol) reacted at  $80^\circ\text{C}$  in ethanol (50 ml) and in the presence of piperidine (5.05 mmol) to yield the benzenethiolato copper(I) polymer.  $(\text{C}_6\text{H}_5\text{CuS})_n$ , calc. C: 41.72, H: 2.91, S: 18.56; found C: 42.39, H: 3.09, S: 17.77.

#### *Bis*(tetramethylammonium)- and *Bis*(tetrabutylammonium)hexa(benzenethiolato)tetracuprate(I), $(\text{Me}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$ 5 and $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$ , 6

Thiophenol (9.71 mmol) and piperidine (9.71 mmol) were dissolved in ethanol (130 ml) and  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (1.71 mmol) in 30 ml acetone was added dropwise. After addition of an ethanolic solution of either tetramethylammonium chloride (6.20 mmol in 20 ml) or tetrabutylammonium bromide (6.20 mmol in 20 ml), pale yellow crystals grew

within 3–5 days. 5,  $\text{C}_{44}\text{H}_{54}\text{Cu}_4\text{N}_2\text{S}_6$ , calc. C: 49.98, H: 5.12, N: 2.65, S: 18.19; found C: 49.87, H: 5.32, N: 2.66, S: 18.51. 6,  $\text{C}_{68}\text{H}_{102}\text{Cu}_4\text{N}_2\text{S}_6$ , calc. C: 58.58, H: 7.37, N: 2.01, found C: 58.06, H: 7.44, N: 2.01.

#### *Bis*(triethylammonium)hexa(benzenethiolato)tetracuprate(I), $(\text{Et}_3\text{NH})_2[\text{Cu}_4(\text{SPh})_6]$ , 7

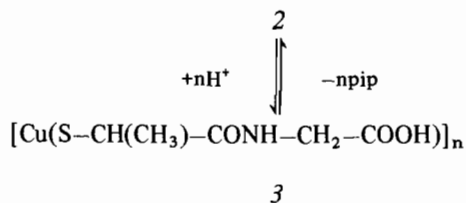
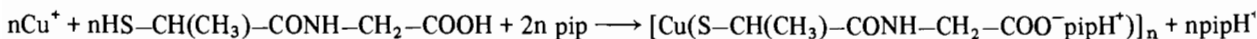
To a stirred solution of thiophenol (4.85 mmol) and triethylamine (4.85 mmol) in ethanol (80 ml) a solution of  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (0.83 mmol) in acetone (20 ml) was added within 60 minutes. When this mixture was stored at room temperature, light yellow crystals started to grow after 24 h and continued to grow for 5 days.  $\text{C}_{48}\text{H}_{62}\text{Cu}_4\text{N}_2\text{S}_6$ , calc. C: 51.77, H: 5.61, N: 2.51; found C: 51.99, H: 5.73, N: 2.64.

#### *Bis*(tetrabutylammonium)hexa(*p*-fluorobenzenethiolato)tetracuprate(I), $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{p-F-PhS})_6]$ , 8

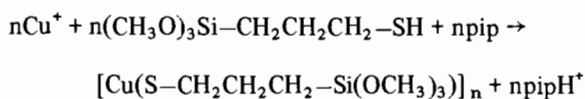
To an ethanolic solution (120 ml) of *p*-fluorothiophenol (10.0 mmol) and piperidine (10.0 mmol),  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (1.66 mmol) in acetone (30 ml) and tetrabutylammonium bromide (6.20 mmol) in ethanol (20 ml) were added. Addition of water (7 ml) to the yellow solution and storage at  $4^\circ\text{C}$  led to crystallization of white microcrystalline  $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{p-F-PhS})_6]$ .  $\text{C}_{68}\text{H}_{96}\text{Cu}_4\text{F}_6\text{N}_2\text{S}_6$ , calc. C: 54.37, H: 6.44, N: 1.86; found C: 54.54, H: 6.66, N: 1.94.

## Results and Discussion

Potentiometric studies on aqueous cuprous mercaptide complexes resulted in polymeric Cu(I)-thiolate species of a 1 Cu:1 SR stoichiometry [8]. A 2 Cu:3SR stoichiometry was proposed as a possible intermediate complex. In Cu–thionein a 1 Cu:2S stoichiometry is known; the copper is exclusively coordinated to sulphur. In this naturally occurring copper protein spontaneous disproportionation of Cu(I) is avoided due to the substantially diminished aprotic environment of the involved cysteines and/or neighbouring aliphatic amino acid residues. It was attempted to mimic this type of copper coordination using aliphatic *N*-2-mercaptopropionylglycine in aprotic solvents. The carboxylate group was chosen in order to discontinue polymerization and to facilitate the formation of the expected tetranuclear  $\text{Cu}_4\text{S}_8$  complex. Piperidine served as a suitable cation. Unfortunately neither the piperidinium salt nor the free carboxylate group were able to inhibit polymerization. The reaction scheme is summarized below.



3-Trimethoxysilylpropane-1-mercaptane was alternatively chosen to minimize polymerization attributable to the large trimethoxysilyl residue. However, only polymeric thiolatocopper(I) species were obtained:



1

In order to compensate for the high negative charge on the four surrounding thiolate sulphurs of a possible  $\text{Cu}_4\text{S}_4$  tetrahedron, a neutral sulphur ligand was additionally employed. Thiourea is well known to form endless  $\text{Cu(I)(SC(NH}_2\text{)}_2)_3$  tetrahedral chains [9]. Equimolar mixtures including the above mercaptanes and thiourea as well as a threefold excess of these ligands resulted in the formation of the polymeric Cu(I) mercaptide complexes described above.

Generally, aliphatic mercaptanes failed to yield the desired tetranuclear complexes, but formed polymeric  $[\text{CuSR}]_n$  species exclusively. Apparently, conditions imposed on Cu(I) coordination by the arrangement of 8 cysteine residues inside the protein moiety are not appropriately mimicked by using comparatively simple models such as N-2-mercapto-propionylglycine.

Formation of oligonuclear clusters requires coordination of more than two thiolates to each copper (see Fig. 1); this in turn is facilitated by  $\pi$ -accepting ligands, e.g. thiophenols [10]. As there are already several different Cu(I) arenemonothiolate complexes known [11, 12], this type of ligand was selected to prepare a suitable Cu(I) tetranuclear compound.

$[\text{CuSR}]_n$  polymers are formed with thiophenols, too. Intriguingly, with  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  (0.02 mol/l) and piperidinium thiophenolate (2-fold excess) in ethanol,  $[\text{CuSPh}]_n$  4 precipitates from warm solutions ( $>40^\circ\text{C}$ ) only, and is isolated quantitatively when filtered near the boiling point. However, upon cooling to room temperature, the microcrystalline  $[\text{CuSPh}]_n$  is readily dissolved, provided there is excess thiophenolate present. Presumably, this is due to  $[\text{Cu}_4(\text{SPh})_6]^{2-}$  complexes being formed, since the addition of tetraalkylammonium halides results in crystallization of  $(\text{R}_4\text{N})_n[\text{Cu}_{2n}(\text{SPh})_{3n}]$ ,

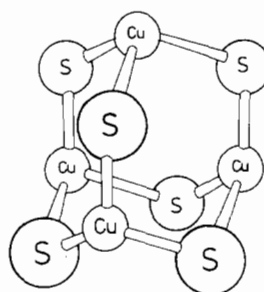
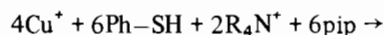


Fig. 2. Pseudo-adamantane  $\text{Cu}_4\text{S}_6$  arrangement of  $[\text{Cu}_4(\text{SPh})_6]^{2-}$ , drawn after Dance and Calabrese [11] (benzene rings omitted).

probably with  $n = 2$  (see below), i.e.:  $(\text{R}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$ .



In contrast to the above reaction employing  $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$  with thiophenol, Dance [11] has approached the synthesis of the  $(\text{Me}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$  using  $\text{Cu}(\text{NO}_3)_2$ . His X-ray structure elucidation revealed a pseudoadamantan array (Fig. 2).

It was hoped to alter the Cu-thiophenolate stoichiometry by the use of larger cations including both tetrabutylammonium and triethylammonium salts. However the same 2 Cu:3 SPh stoichiometry was observed. Recently, Coucouvanis *et al.* [13] reported similar results using  $\text{Ph}_4\text{P}^+$  as the cation. Likewise, even the enhanced  $\pi$ -acidity of *p*-fluorothiophenol did not alter the Cu:S ratio;  $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{p-F-SPh})_6]$  8 was isolated under similar conditions. Throughout, the tetranuclear  $[\text{Cu}_4(\text{SPh})_6]^{2-}$  or  $[\text{Cu}_4(\text{p-F-SPh})_6]^{2-}$  species were obtained. It may be concluded that this is the thermodynamically most favourable arrangement. Nevertheless, a 1 Cu:1.5 S stoichiometry is getting closer to the 1 Cu:2 S ratio normally present in Cu-thionein.

TABLE I. Infrared Spectral Data of the Tetranuclear Complexes 5–8 ( $\text{cm}^{-1}$ , in KBr).<sup>a</sup>

	$\nu(\text{CH})_{\text{arom.}}$	$\nu(\text{CH})_{\text{aliph.}}$	$\nu(\text{C}=\text{C})_{\text{arom.}}$	$\nu(\text{CS})$	$\nu(\text{CF})$	$\nu(\text{CuS}) ?$
5 $(\text{Me}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$	3060m 3015w	2920w	1565s 1470s	895w		203 <sup>b</sup> 273 350
6 $(\text{Bu}_4\text{N})_2[\text{Cu}_2(\text{SPh})_6]$	3050s	2960s 2940s 2870s	1570s 1475s	885m		200 ? 230 335
7 $(\text{Et}_3\text{NH})_2[\text{Cu}_4(\text{SPh})_6]$	3070m	2980m	1565s 1465s	890m		265 330
8 $(\text{Bu}_4\text{N})_2[\text{Cu}_4(p\text{-F-SPh})_6]$	3060w 3030w	2960s 2940s 2870s	1570m 1480s	875w	820s	210 230 310

<sup>a</sup>s = strong; m = medium; w = weak. <sup>b</sup>Ref. 15.

TABLE II. <sup>1</sup>H NMR Data of Compounds 5–8 (in  $d_6$ -acetone, in ppm).

5 $(\text{Me}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$	$\text{C}_6\text{H}_5$ : 7.5–6.6 (m, (15H)n); $\text{CH}_3$ : 3.23(s, (12H)n)
6 $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$	$\text{C}_6\text{H}_5$ : 7.5–6.6 (m, (15H)n); N–H: 7.35(s); $\text{CH}_2$ : 3.07(q, 7.04 Hz, (6H)n); $\text{CH}_3$ : 1.16(t, (16H)n); $\text{CH}_3$ : 0.92 (t, 7.04 Hz, (12H)n)
7 $(\text{Et}_3\text{NH})_2[\text{Cu}_4(\text{SPh})_6]$	$\text{C}_6\text{H}_5$ : 7.5–6.6 (m, (15H)n); N–H: 7.35(s); $\text{CH}_2$ : 3.07(q, 7.04 Hz, (6H)n); $\text{CH}_3$ : 1.16(t, 7.19 Hz, (9H)n)
8 $(\text{Bu}_4\text{N})_2[\text{Cu}_4(p\text{-F-SPh})_6]$	$\text{C}_6\text{H}_4$ : 7.5–6.3 (m, (12H)n); $\text{CH}_2$ : 3.31 (t, 7.35 Hz, (8H)n); $\text{CH}_2$ – $\text{CH}_2$ : 1.8–1.2 (m, (16H)n); $\text{CH}_3$ : 0.93 (t, 6.9 Hz, (12H)n)

### Infrared Spectra

Infrared spectroscopy was carried out to obtain some information regarding the copper sulphur binding (Table I).

Both for the polymeric cuprous mercaptanes and the tetranuclear copper(I) thiophenolates, loss of the  $\nu(\text{SH})$  absorption indicates Cu–S bond formation.

As regards the  $[\text{CuSR}]_n$  polymers, the ir spectra closely resemble those of the free mercaptanes. The mercaptopropionylglycine derivatives exhibit the characteristic absorptions of a carboxylic acid (3,  $\nu(\text{CO})$  1715  $\text{cm}^{-1}$ ) or carboxylate group (2,  $\nu(\text{CO})$  1590  $\text{cm}^{-1}$ ), respectively. Contrary to the corresponding Cu(II) complex there is no indication of chelate formation, *i.e.* coordination of the amide nitrogen, since this would be accompanied by a shift of the amide-II band to higher wavenumbers [14] and probably by the loss of the  $\nu(\text{NH})$  mode.

Vibrational spectra for tetranuclear Cu(I) thiophenolate complexes have not yet been reported,

except for far infrared and low frequency Raman data of  $(\text{Me}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$  [15]. The ir pattern of these complexes is essentially the sum of the contributions assigned to the respective ammonium ion plus those of the ligand (Table I). The shift observed for the  $\nu(\text{CS})$  band, however, merits further comment. The thiophenolic C–S bond achieves a partial  $\pi$ -character through electron delocalization. Thus, the  $\nu(\text{CS})$  is found at 850–920  $\text{cm}^{-1}$ , *i.e.* between absorptions typical for a C–S single (around 700  $\text{cm}^{-1}$ ) and a C=S double bond (1050–1200  $\text{cm}^{-1}$ ), respectively. Frequencies with at least partial  $\nu(\text{CS})$  character of both thiophenol and *p*-fluorothiophenol are located at 905  $\text{cm}^{-1}$ . Upon coordination to Cu(I), these bands are shifted to 875–890  $\text{cm}^{-1}$  (Table I). We suggest that  $d_{\pi-\pi^*}$  back-donation occurs from Cu to S, leading to weakening of the C–S  $\pi$ -bond in the order of 20–40 wave numbers.

As for the Cu–S stretch, no unequivocal assignments have been made so far; they are, however, assumed to appear near and below 300  $\text{cm}^{-1}$  [16],

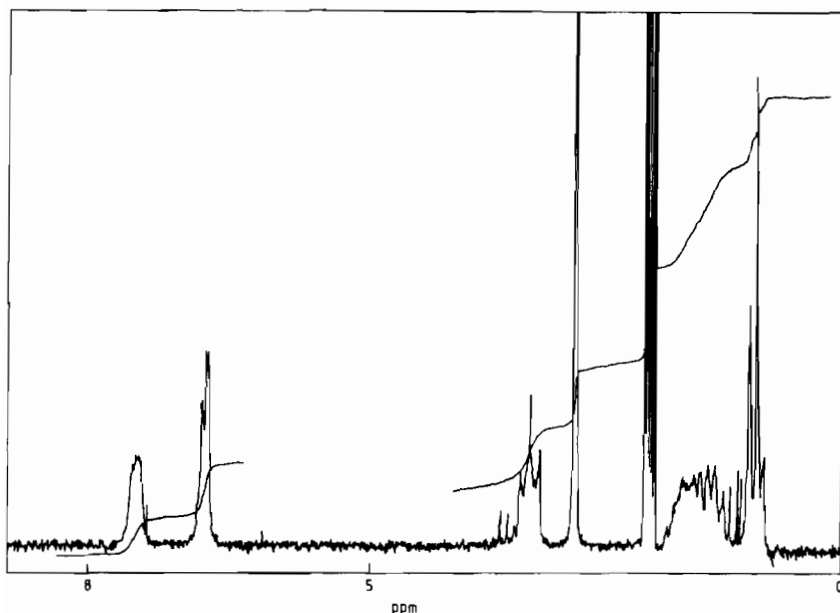


Fig. 3.  $^1\text{H}$  nmr of  $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$  7 (in  $d_6$ -acetone).

together with breathing modes of the  $\text{Cu}_4\text{S}_6$  unit. Vibrational analysis predicts two  $\nu(\text{CuS})$  and two cage deformation bands of the  $T_d$   $\text{Cu}_4\text{S}_6$  moiety (Fig. 2) [15]. 'Less symmetric' oligonuclear species would lead to a much higher number of absorptions near and below  $300\text{ cm}^{-1}$ . Our observation of only 3 bands in this region (around 200, 260 and  $310\text{ cm}^{-1}$ ) strongly supports our suggestion of a tetranuclear complex of  $T_d$  symmetry. However, another highly symmetric arrangement, *e.g.*, a  $\text{Cu}_8\text{S}_{12}$  species of cubic  $\text{Cu}_8$  arrangement with edge-bridging sulphurs cannot be excluded. Cu-S stretching modes produce inherently weak IR absorptions. Thus, they are not observable for yeast copper-thionein itself.

#### $^1\text{H}$ Nmr Spectra

Nmr spectra are readily obtained for all four tetranuclear complexes, *i.e.* with all copper being Cu(I). Again, this parallels the situation observed for copper metallothioneins [2]. The relevant data are listed in Table II; the spectrum of  $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$  is depicted in Fig. 3. Generally, coordinated thiophenol gives rise to resonances at 6.6–7.5 ppm (6.3–7.5 ppm for *p*-fluorothiophenol). Peaks around 3.3 ppm ( $\alpha\text{-CH}_2$  or  $\alpha\text{-CH}_3$ ) and 0.9–1.8 ppm are assigned to the respective aliphatic ammonium ions. Integration is conclusive for the stoichiometric ratio of ammonium ion to thiophenolate, which turns out to be exactly 1:3 for all compounds (Table II). Hence, for complexes containing copper(I) a composition of  $(\text{R}_4\text{N})_n[\text{Cu}_{2n}(\text{SPh})_{3n}]$  has to be assumed.

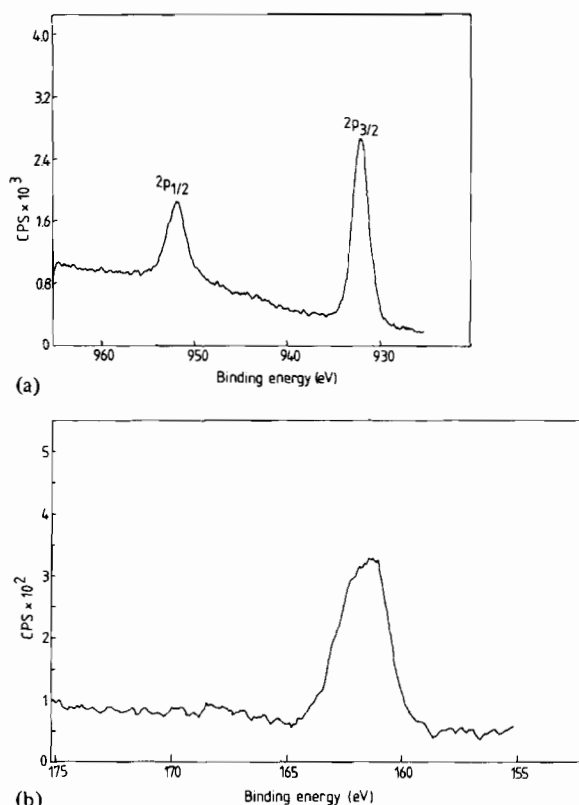


Fig. 4. X-ray photoelectron spectra of  $(\text{Bu}_4\text{N})_2[\text{Cu}_4(\text{SPh})_6]$  6: (a) Cu  $2p_{1/2}$  and Cu  $2p_{3/2}$  levels, (b) S  $2p_{(1/2, 3/2)}$  levels. Recording conditions: Excitation energy Mg  $K\alpha$  1253.6 eV. Pressure range  $\leq 10^{-7}$  mbar. Pass energy  $150\text{ eV} \pm 1.4\text{ eV}$ . Number of scans 9. Sweep time 4.5 sec, sweep width 20–50 eV. Temperature  $-150^\circ\text{C}$ .

*X-ray Photoelectron Spectrometry*

The actual charge on both copper and sulphur of the  $[\text{Cu}_4(\text{SPh})_6]^{2-}$  cluster was measured to compare this copper complex with copper–thionein. In Cu–thionein the binding energy of the Cu  $2p_{3/2}$  core electrons is near 932.2 eV [3]. Owing to the absence of any satellites, the copper was assigned to be in the  $3d^{10}$  state. The binding energy value of the unresolved S  $2p_{(1/2,3/2)}$  doublet was at 162.0 eV. Virtually identical values were seen when Cu(I)-(thiourea)<sub>3</sub>Cl was measured (Cu  $2p_{3/2}$  = 932.5 eV, S  $2p_{(1/2,3/2)}$  = 162.2 eV). It was intriguing to note an even closer relationship between the binding energy values of the core electrons of copper and sulphur in both Cu–thionein and  $[\text{Cu}_4(\text{SPh})_6]^{2-}$ . The sulphur values are identical at 162.0 eV. There is only one thiolate sulphur signal suggesting the purity of the sample and the chemical equivalence of all involved sulphur atoms (Fig. 4a). The Cu  $2p_{3/2}$  value at 932.3 is only 0.2 eV different from the Cu–thionein value (Fig. 4b). The absence of any satellites again supports the  $3d^{10}$  electronic state of the copper. The binding energy value of the Cu  $2p_{1/2}$  level at 951.9 eV is consistent with earlier data of Cu(I) complexes [17].

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