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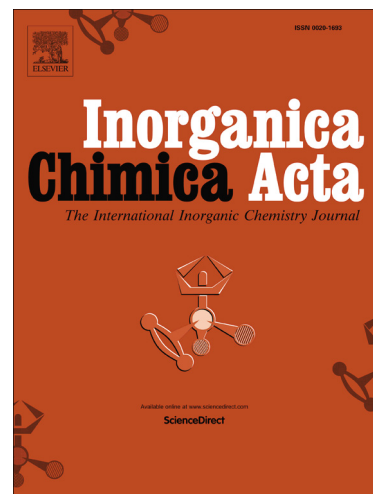
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Metal complexation Induces Antibiotic Activity in S-ethyl-L-cysteine Sulfoxide

Shahbaz Nazir*^a, Jamil Anwar^a, Munawar Ali Munawar^a, Javed Iqbal Qazi^b, Stephen Peter Best^c, Michael Cheah^c, Muhammad Yaseen^d.

^a Institute of Chemistry, University of the Punjab, Lahore 54590, Pakistan

^b Department of Zoology, University of the Punjab, Lahore 54590, Pakistan

^c School of Chemistry, University of Melbourne, Melbourne, Australia..

^d Department of Chemistry, University of Education, Faisalabad campus, Faisalabad, Pakistan.

Anti-microbial effect is produced when the ligand (L) s-ethyl-L-cysteine sulfoxide (Ethiin) makes coordination complex with metal 'M' (M= Cu, Co, Ni), while, ethiin did not show any antimicrobial effect. The synthesized complexes have 1:2 metal to ligand ratio (ML₂). The spectro-chemical analysis revealed that the ligand coordinates with the metals through oxygens of the carboxylate and sulfoxide groups resulting in octahedral geometry around the central metal atom. Techniques like ¹H-NMR, thermal analysis, mass spectrometry, UV-Vis spectroscopy, XAFS, FTIR, EPR and magnetic moments were used to elucidate the structure of the ligand and the metal complexes. The expected reasons for the creation of anti-microbial effect are, either the disturbance of the ionic environment within the microbial cytoplasm when the complex enters into it or interaction of the complex with the DNA.

Keywords: S-ethyl-L-cysteine sulfoxide complexes, antimicrobial effects, sulfoxide coordination complex, XAFS analysis

1. Introduction

Allium plants (Onion, Garlic, Shive etc.) are rich source of sulphur containing compounds, that mostly, consist of cysteine derivatives *e.g.*, S-Allyl cysteine, S-Ethyl cysteine, S-Propyl cysteine and their sulfoxides [1,2]. These compounds have variety of health effects such as anticarcinogenic, antibiotic, anticoagulant, antiatherogenic, antihyperlipidaemic, antihypertensive, immune system protection / stimulant, hepatic system protection, antidiabetic and antioxidant effects [3, 4-15]. For example, S-propyl cysteine, S-propyl cysteine sulfoxide and S-methionine sulfoxide reduce the risk of atherosclerosis [16, 17]. The S-alk(en)nyl-L-cysteines *e.g.*, Methyl cysteine, Allyl cysteine and their sulfoxides show strong antioxidant activity when tested against lipid hydroperoxide (LOOH) formation in human low density lipoproteins (LDL)[18]. Not only these compounds, but their metal complexes also show promising health effects. For example, Corbi *et al.* prepared platinum complex of S-allyl-L-cysteine sulfoxide (alliin). This complex revealed moderate cytotoxic effect against human cancer cell line HeLa [19]. Corbi *et al.* also prepared palladium complex of methionine sulfoxide. This complex exhibited a high cytotoxic effect against human cancer cell line HeLa [20].

*Corresponding author. Email: shahbaznazir2000@yahoo.com

S-ethyl-L-cysteine sulfoxide (Fig. 1) is an important molecule found in *Allium* plants. Considering the bioactive potential of cysteine sulfoxides and their metal complexes, we have prepared the metal complexes of S-ethyl-L-cysteine sulfoxide and studied their biological activity.

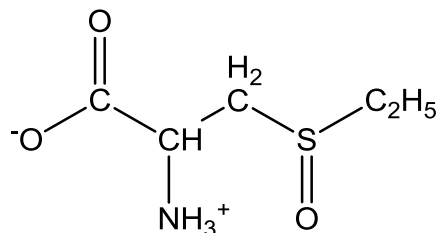


Fig. 1: S-ethyl-L-cysteine Sulfoxide

2. Experimental

2.1 Material, Methods and Equipment

Melting points of the ligand and its metal complexes were determined using a Gallenkamp melting point apparatus. ^1H NMR spectrum was taken in D_2O and recorded at 500 MHz on Varian INOVA-500 instrument. (Abbreviations: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet).

For ^{13}C NMR spectra, solution of Ethiin in D_2O was added with acetone as internal standard.

Mass spectrum was recorded on Jeol MS Route and Waters micromass triple quadrupole quarto II Electropray Ionization mass spectrometer. Cone voltages ranging from 5-15 V were used for best results. IR spectra were recorded on FT-IR Nicolet 6700 of Thermo Scientific Company. Elemental analysis was performed using CHNS-932 LECO analyzer. UV-Vis spectra were recorded from 200-800 nm by using Spectro UV-Vis double beam UVD-3500 (Labomed INC.). Magnetic moments were measured at 20°C using Mark 1 instrument from Sherwood scientific company. Mercury tetrathiocyanatocobaltate (II) $\{\text{Hg}[\text{Co}(\text{NCS})_4]\}$ was used as a calibrant. TGA, DTA were taken by using Universal V4.2E TA instrument-SDT Q600 V8.2 Build 100 and analysis were made in Nitrogen environment with heating rate of $10^\circ\text{C min}^{-1}$ from 25 to 1000°C . Reagent grade materials were purchased from Aldrich Chemical Co. and used without further purification.

X-ray absorption spectra were collected from solid samples of copper complexes of S-propyl-L-cysteine diluted into cellulose powder and finely ground and pressed into 13mm discs by the application of moderate pressure (10 Tonnes). The composition of the disc was adjusted so as to achieve a balance between the attenuation of the sample and the transmittance beyond the Cu absorption edge. For all samples the minimum transmittance was between 10 and 20% through the edge and XAFS regions.

X-ray fluorescence measurements were made at a temperature of ca. 273K using beamline 20 B (bending magnet) at the KEK Photon Factory, Tsukuba, Japan. A channel-cut Si(1 1 1) monochromator provided an energy resolution (DE/E) of ca. 2.4×10^{-4} , and higher order harmonics of the selected wavelength were rejected by detuning the monochromator by a factor of a half. EXAFS measurements were conducted at the Cu K-edge. Solid samples were measured in transmission mode using an ion chamber detector. Data reduction of experimental X-ray

absorption spectra was performed using the program XFIT [21]. The model structure was refined using the XFIT software package, which incorporates ab initio FEFF 6.01MS curved wave calculations [22]. The number of refined variables, Nrv, in the models, including the scale factors E0 and S0 2 are listed in appendix.

2.2 Synthesis of S-alkyl-L-cysteine sulfoxide

S-ethyl-L-cysteine sulfoxide was prepared using slightly modified method of Tsuge *et al.* [23]. Hence, S-ethyl-L-cysteine (8.25g, 0.1 mole) was dissolved in 500 ml of distilled water and 10 ml of 30% hydrogen per oxide were added to it drop by drop. The resulting solution was stirred for 2 hours and concentrated under reduced pressure. The product was crystallized from the solution by the addition of acetone (1000 ml) and recrystallized from ethanol/water system.

Yield 79 %. Anal. Cald. for $(C_5H_{11}NO_3S)$ (%): C, 36.36; H, 6.66; N, 8.48; S, 19.39. Found: C, 36.31; H, 6.45; N, 8.58; S, 19.28. m.p. 160-161 °C (dec.); IR (cm^{-1}): 1588 ($\nu_{(COO^-)}$), 3180-2865 ($\nu_{(NH_3^+)}$), 1014 ($\nu_{(S=O)}$). ESI-MS m/z : 166.2 $[M+H]^+$; 1H NMR (D_2O , PPM, 500 MHz): δ 4.21 (d of t, $J=6.0$ and 6.5 Hz, 1H, H at α -Carbon), 3.45 (dd, $J=7.5$ and 14 Hz, 1H, Ha on β -carbon), 3.18 (dd, $J=6.0$ and 14 Hz, 1H, Hb on β -carbon), 2.92 (m, 2H, CH_2S on ethyl side), 1.29 (t, $J=7.5$ Hz, 3H, CH₃). ^{13}C NMR (D_2O , ppm, 125 MHz): δ 173.0, 51.6, 51.2, 46.9, 6.50.

2.3 Synthesis of S-Ethyl-L-cysteine sulfoxide (Ethiin) complexes

Ethiin (3.3g, 0.02 mole) was dissolved in 0.02 molar ethanolic solution of sodium hydroxide. MCl_2 hydrate ($M= Cu, Co, Ni$) (0.01 mole) was dissolved in minimum amount of water and added in the ethiin solution drop by drop and stirred vigorously for an hour. Gel like precipitates having colour characteristics of respective metal complexes were filtered, washed with ethanol thoroughly and dried in oven at 50°C to obtain solid mass.

2.3a Bis(S-Ethyl-L-cysteine sulfoxidato)Cu(II): $[Cu(C_5H_{10}NO_3S)_2]$

Yield 82 %. Anal. Cald. for $[Cu(C_5H_{10}NO_3S)_2]$ (%): C, 3.65; H, 5.10; N, 7.15; S, 16.34. Found: C, 30.31; H, 5.19; N, 7.23; S, 16.28. m.p. Dec. >180 °C; FT-IR (cm^{-1}): 3288, 3247 ($\nu_{(NH_2)}$), 1596, 1398 ($\nu_{(COO^-)}$), 1017 ($\nu_{(S=O)}$). UV-Vis (cm^{-1}): 15923. μ_{eff} (B.M.): 2.12.

2.3b Bis(S-Ethyl-L-cysteine sulfoxidato)Co(II): $[Co(C_5H_{10}NO_3S)_2]$

Yield 85 %. Anal. Cald. for $[Co(C_5H_{10}NO_3S)_2]$ (%): C, 31.00; H, 5.16; N, 7.23; S, 16.53; Found: C, 31.17; H, 5.25; N, 7.35; S, 16.64. m.p. Dec. >180 °C; FT-IR (cm^{-1}): 3347, 3281 ($\nu_{(NH_2)}$), 1592, 1410 ($\nu_{(COO^-)}$), 1014 ($\nu_{(S=O)}$). ESI-MS $[M+H]^+$: 388.1. UV-Vis (cm^{-1}): 37040, 19723. μ_{eff} (B.M.): 5.24

2.3c Bis(S-Ethyl-L-cysteine sulfoxidato)Ni(II): $[Ni(C_5H_{10}NO_3S)_2]$

Yield 78 %. Anal. Cald. For $[Ni(C_5H_{10}NO_3S)_2]$ (%): C, 31.03; H, 5.17; N, 7.24; S, 16.55; Found: C, 31.18; H, 5.28; N, 7.36; S, 16.65. m.p. Dec. >182 °C; FT-IR (cm^{-1}): 3346, 3274 ($\nu_{(NH_2)}$), 1584, 1397 ($\nu_{(COO^-)}$), 1016 ($\nu_{(S=O)}$). UV-Vis (cm^{-1}): 27100, 16077. ESI-MS $[M+H]^+$: 387.4. μ_{eff} (B.M.): 3.32

3. Results and Discussion

3.1 FTIR spectroscopy

S-ethyl-L-cysteine sulfoxide (Fig. 2) can coordinate with metals through oxygen, sulfur and nitrogen atoms (in bold).

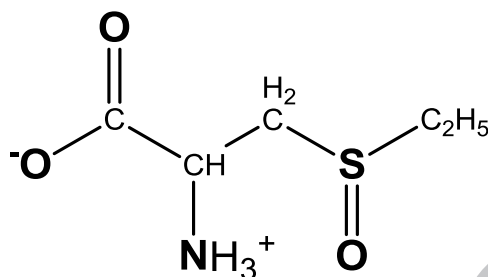


Fig. 2: Donor atoms (in bold) of S-ethyl-L-cysteine Sulfoxide

Since amino acids exist in zwitter ionic form, so depending on the pH and metal atom Ethiin can act as monodentate, bidentate and tridentate ligand and can be a bridging or non-bridging ligands [24, 25, 26].

Literature values for vibrational frequencies of various groups of amino acids, potassium salt of an amino acid for reference and Ethiin are given in table 3.1.

Table 3.1

Literature values for vibrational frequencies of various groups of amino acids, potassium salt of amino acids and the ligands. (cm^{-1})

Amino Acid	$\nu_a \text{NH}_3^+$	$\nu_\delta \text{NH}_3^+$	$\nu_{as} \text{COO}^-$	$\nu_s \text{COO}^-$	S=O	Ref.
General trend	3130-2500 (broad-band)	1660-1610 1550-1485	1600-1560	~1410		28
Alanine	3180-2510	1520	1575	1415		36
Methionine Sulfoxide	3200-2700	1515	1585	1414	1021	29
Methionine sulfoxide Potassium salt	3600-2750		1587	1410	1020	29
S-ethyl-L-cysteine sulfoxide	3210-2666	1506	1596	1398	1014	

In solid state, amino acids exist in Zwitter ionic form and hence they show different IR pattern than the normal amino (NH_2) and carboxylic (COOH) group. They show IR pattern characteristic of ionic carboxylate and amine salt. The broad band of NH_3^+ is composed of asymmetric and symmetric stretches, combination and overtones overlapping with each other [27]. This region becomes wider due to participation of C-H stretching vibrations of the amino acid. This region become even wider in ionic salts of the amino acids as NH_3^+ is deprotonated here and N-H vibrational frequencies rise due to increase of N-H bond strength. Furthermore hydrogen bonding involved also contribute in the band widening [28].

Similarly ionized carboxylic group either in zwitter ionic form or in basic salt form *e.g.*, potassium salt, show absorption band due to asymmetric and symmetric (COO^-) stretches around 1580 and 1410 cm^{-1} respectively. The protonated carboxylic group *e.g.*, in hydrochloride salt absorb between 1700-1750 cm^{-1} [27,28].

In case of amino acids containing sulfoxide ($\text{S}=\text{O}$) group, IR absorption for sulfoxide group appears as strong band around 1020 cm^{-1} [29].

On coordination of the amino acids to the metal, following changes can be observed in IR absorption frequencies for amino (NH_2), carboxylic (COO^-) and sulfoxide ($\text{S}=\text{O}$) groups.

1. Coordination of amino group is attested by the presence of two well resolved peaks in the region 3000-3400 cm^{-1} . Amino group coordination is also confirmed by the absence of band in the region 2050-2150 cm^{-1} . This band is present in free amino acid and their basic salts and is assigned to non-coordinated NH_2 group [30]. The absence of $\delta_{\text{as}}(\text{NH}_3^+)$ band in the complex is another proof of NH_2 coordination. In free ligand this band is present at 1505 cm^{-1} [28].
2. Coordination of carboxylate (COO^-) group to metal causes the asymmetric frequency to increase and symmetric frequency to decrease. The amount of increase and decrease depends on the metal involved which in turn depends upon the strength of M-O bond. The stronger the M-O bond, the more is the increase in asymmetric frequency [28].
3. For sulfoxide coordination, it is necessary to consider the structures of hybrids of sulfoxide which are shown in Fig. 3, [28].

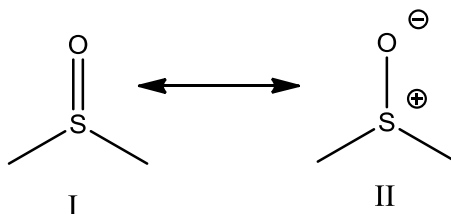
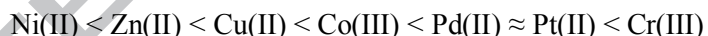


Fig. 3: Hybrid structure of sulfoxide group

Sulfoxide group can coordinate either through sulfur atom or through oxygen atom. If coordination occurs through oxygen atom, it will give rise to partially ionized bond and will make contribution of structure (I) weaker resulting in weakening of (S=O) bond and hence its frequency will be shifted to lower value.

On the other hand if coordination occurs through the sulfur atom, the contribution of structure (II) will decrease resulting in strengthening of (S=O) bond and hence the frequency of absorption will increase [28].

Another parameter for the metal complexes is $\Delta\nu$ which is difference of asymmetric and symmetric vibration frequency of carboxylate group of the amino acid, hence $\Delta\nu = \nu_{as} - \nu_s$ (COO^-). When an amino acid coordinates, the asymmetric vibration frequency increases and symmetric vibration frequency decreases as compared to the free ionic state which in turn depends on the metal oxygen (M-O) bond strength. The stronger the M-O bond, the more is the shift in asymmetric and symmetric frequencies. Nakamoto gave the following order of metals for the increase in the separation of the two frequencies on coordination [28].



The $\Delta\nu$ not only tells about the strength of the M-O bond but it also tells about the mode of the coordination of the carboxylate group. Carboxylate group can coordinate either in monodentate fashion (only one oxygen coordinating) or bidentate fashion (both the oxygen involved in coordination). The monodentate coordination gives rise to higher $\Delta\nu$ values while bidentate coordination gives rise to $\Delta\nu$ closer to ionic carboxylate group e.g. in potassium salts of the amino acids [28].

IR frequencies for metal-oxygen and metal-nitrogen bond are also an important proof for coordination of the ligands with metals. For the metals of first transition series in +2 oxidation state, the M-N stretching frequency occurs between $400\text{-}500\text{ cm}^{-1}$ and M-O frequency occurs between $280\text{-}400\text{ cm}^{-1}$. These frequencies decrease as atomic radius of the metal involved increases and vice versa [28].

Selected peaks of IR spectrum of the S-ethyl-L-cysteine sulfoxide complexes are given in table 3.2.

Table 3.2

IR data of S-ethyl-L-cysteine sulfoxide complexes

Complex	NH ₃ ⁺ /NH ₂ (cm ⁻¹)	v _{as} COO ⁻ (cm ⁻¹)	v _s COO ⁻ (cm ⁻¹)	Δv = v _{as} - v _s (COO ⁻)	S=O
Ethiin Potassium salt	3210-2666 (broad band)	1589	1395	194	1014
Cu ethiin	3398-3147 (broad band)	1631	1393	238	1002
Co ethiin	3365-3181 (broad band)	1592	1410	182	1004
Ni ethiin	3356-3174 (broad band)	1594	1405	189	1006

In all the complexes, a broad band in the region 3400-3100 indicates a non-coordinated amino group. So coordination of the amino group is ruled out.

The changes in the carboxylate group frequencies prove its coordination to metals. The Δv clearly indicates that carboxylate group is coordinated in bidentate fashion. This coordination is symmetric as both the antisymmetric and symmetric frequency values increase as compared to cases where anti-symmetric increases and symmetric decreases which indicates unsymmetric bidentate coordination [31].

In all the complexes a coordination of sulfoxide group through oxygen atom is predicted as vibrational frequency decreases in complexes as compared to free ligand or its potassium salt.

Metal ligand vibration frequencies for Ni, Co, Cu can be found at 305, 383, 371 cm⁻¹ respectively for Metal-oxygen (M-O) bond of the metal coordinated to carboxylate group .

3.2 Thermogravimetric analysis

Thermogravimetric analysis of the complexes is discussed below.

3.2a Bis(S-ethyl-L-cysteine sulfoxidato)Cu(II): [Cu(C₅H₁₀NO₃S)₂]

In the TGA of copper complex of ethiin, the decomposition proceeds in one step as shown in Fig. 4. Here the entire ligand is removed around 200 °C. DTA shows that this one step is composed of two different heat absorption steps. Hence the two step of decomposition takes

place with a difference of very short time. Final residue was CuO. Hence, the molecular formula was suggested to be $\text{Cu}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$. No coordinated water was detected.

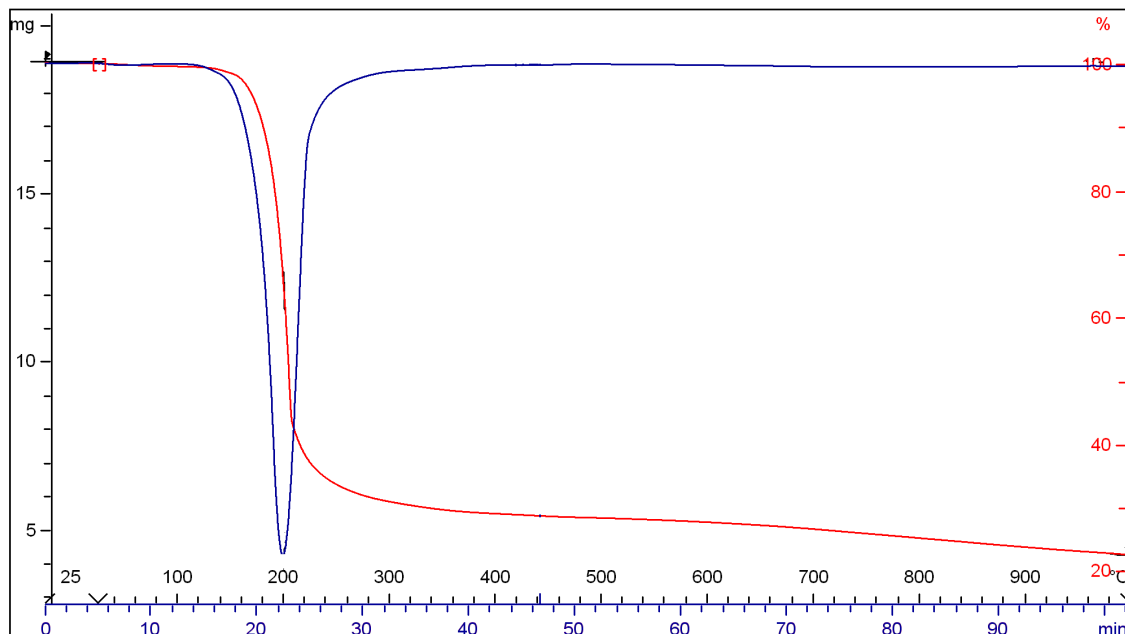


Fig. 4: DTA and DTG of Bis(S-ethyl-L-cysteine sulfoxidato)Cu(II).

3.2b Bis(S-ethyl-L-cysteine sulfoxidato)Co(II): $[\text{Co}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2]$

Decomposition, according to TGA of cobalt ethiin complex, takes place in two distinct steps as shown in Fig. 5. The first step is the removal of carbon chain including alpha carbon i.e. $-\text{CH}-\text{CH}_2-\text{S}(\text{O})-\text{CH}_2-\text{CH}_3$. The second step is removal of the rest of the ligand from the molecule. DTG and DTA curves also confirm the same pattern. Final residue was CoO so the formula was supposed to be $\text{Co}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$. No water of coordination was detected in the spectra.

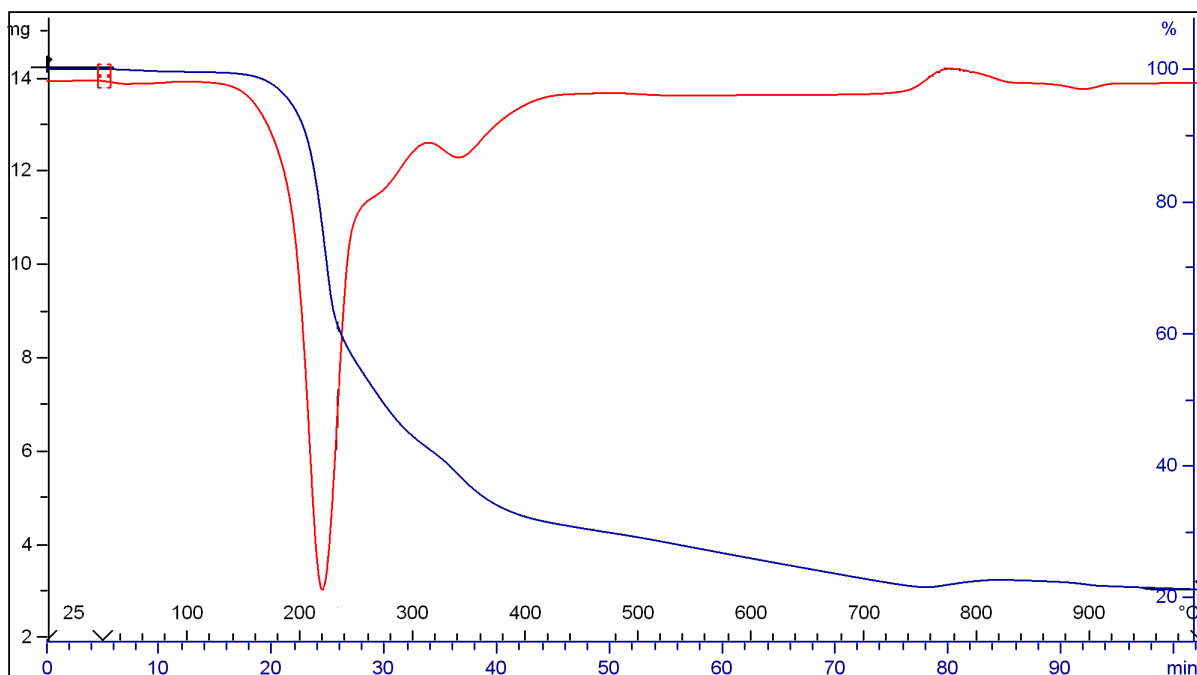


Fig. 5: TGA and DTG of Bis(S-ethyl-L-cysteine sulfoxidato)Co(II).

3.2c Bis(S-ethyl-L-cysteine sulfoxidato)Ni(II): $[\text{Ni}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2]$

TGA of nickel ethiin complex shows single step decomposition around 240 °C as shown Fig.6. All of the ligand is removed in one step. DTG and DTA confirm this observation. Final residue was NiO, hence the molecular formula was supposed to be $\text{Ni}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$. No coordinated water was detected.

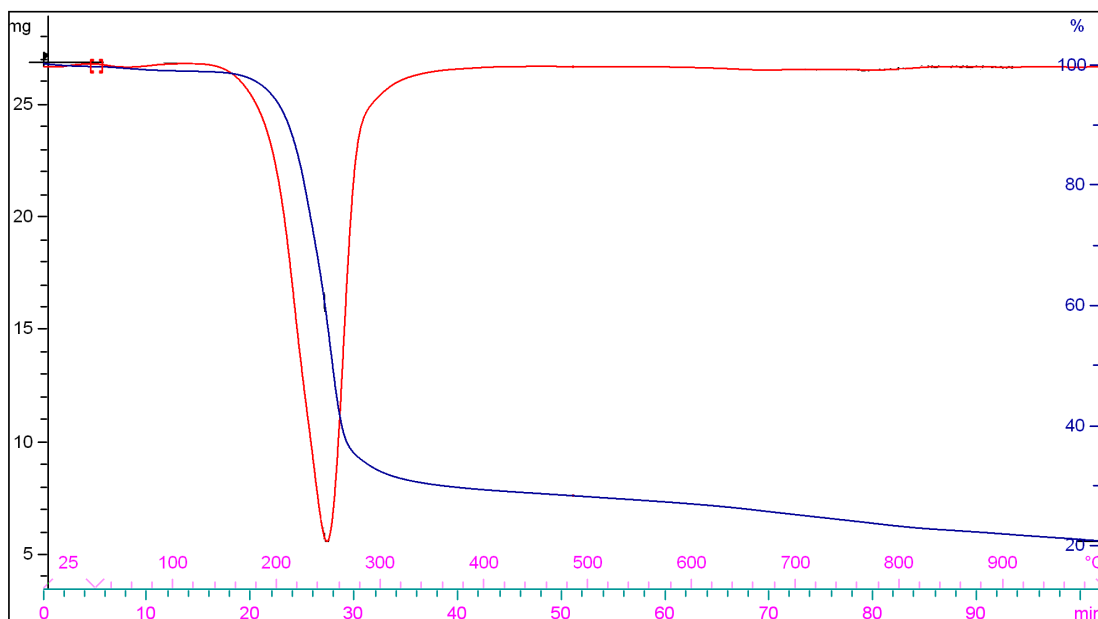


Fig. 6: TGA and DTG of Bis (S-ethyl-L-cysteine sulfoxidato) Ni(II).

3.3 ESI-Mass Analysis

The important peaks in the ESI-Mass spectrum of all the complexes are $[M+H]^+$ peaks. The complexes with proposed molecular formulas and peaks values are as follows,

- i) Bis(S-ethyl-L-cysteine sulfoxidato)Cu(II): $\text{Cu}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$, = 392.7
- ii) Bis(S-ethyl-L-cysteine sulfoxidato)Co(II): $\text{Co}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$, = 388.1
- iii) Bis(S-ethyl-L-cysteine sulfoxidato)Ni(II): $\text{Ni}(\text{C}_5\text{H}_{10}\text{NO}_3\text{S})_2$, = 387.4

These peaks are accompanied with isotopic pattern for the respective metals.

The other important peaks are of monmeric ligand at 166.1 and dimeric ligand at 331.4. These are also accompanied with all the isotopic pattern for organosulfur compound. Peaks of the ligand with S-alkyl group removed are also present.

3.4 Magnetic Momment

Magnetic moments of copper, cobalt and nickel complexes are given in table 3.3.

Table 3.3

Magnetic moment of S-ethyl-L-cysteine sulfoxide complexes

Sr. No.	Complex	μ_{eff}	Limit	Ref.
1	Bis(S-ethyl-L-cysteine sulfoxidato)Cu(II)	2.12	1.7-2.2	37
2	Bis(S-ethyl-L-cysteine sulfoxidato)Co(II)	5.24	4.1-5.2	37
3	Bis(S-ethyl-L-cysteine sulfoxidato)Ni(II)	3.32	2.8-4.0	37

Cobalt, Nickel and copper have d^7 , d^8 , d^9 configuration respectively and here cobalt has high spin configuration. An octahedral geometry is proposed with the support of UV-Vis data for these complexes.

3.5 UV-Vis Spectroscopic Measurements

All of these complexes were soluble in water. Lambda max (λ_{max}) and extinction coefficients are given as in table 3.4.

Table 3.4

UV-Vis. Data of S-ethyl-L-cysteine sulfoxide complexes

Complex	λ_{max} nm	ϵ ($l \text{ mol}^{-1} \text{ cm}^{-1}$)	Band assignment	Ref.
Bis(S-ethyl-L-cysteine sulfoxidato) Cu(II)	622	46	$d_{xy, yz, xz} \longrightarrow d_{x^2-y^2}$	
Bis(S-ethyl-L-cysteine sulfoxidato) Co(II)	507	22	${}^4T_{1g} \longrightarrow {}^4T_{1g}(P)$	
Bis(S-ethyl-L-cysteine sulfoxidato) Ni(II)	1012	18	${}^3A_{2g} \longrightarrow {}^3T_{2g}$	
	617	16	${}^3A_{2g} \longrightarrow {}^3T_{1g}$	37
	369	28	${}^3A_{2g} \longrightarrow {}^3T_{1g}(P)$	

These absorption values are characteristics of octahedral geometry as similar results have been found for other octahedral systems of related copper, cobalt and nickel complexes. [25,32, 33-34]

3.6 EPR Spectroscopy

Electron paramagnetic resonance spectroscopy is an important tool to study the distribution of electron density of an orbital around the metal atom like copper. The tetragonal

distortion of the copper complexes from octahedral geometry due to Jahn-Teller effect is studied through this technique. The value of the g-factor for copper complex is given in Table 3.5.

Table.3.5

Values of g-factor for the synthesized copper complex

Sr. No.	Complex	g_1	g_2
1	Bis(S-ethyl-L-cysteine sulfoxidato)Cu(II)	2.06	2.20

The g_1 and g_2 values for the tetragonally distorted complexes e.g. copper complex of methionine sulfoxide are 2.05 and 2.21 respectively and for $[\text{Cu}(\text{H}_2\text{O})_4(\text{HCO}_2)_2]$ are 2.06 and 2.31 [33]. Similar values have also been reported for the other tetragonally distorted octahedral copper complexes [35].

So comparison of the above tabulated data with the published literature values indicates a tetragonally distorted octahedral geometry due to Jahn-Teller effect for the synthesized copper complex. Hence the copper complex has a tetragonally distorted octahedral geometry.

3.7 XAFS Analysis

Structural parameters obtained from fitting the XAFS of bis(S-ethyl-L-cysteine sulfoxidato)Cu(II) are given in table 3.6.

Table 3.6

Structural parameters obtained from fitting the XAFS of bis(S-ethyl-L-cysteine sulfoxidato)Cu(II)

Model	Model 1	Model 2	Model 3
	$L_x, L_y, L_z = O$	$L_x, L_y = O$ $L_z = N$	$L_x, L_y = O$ $L_z = S$
Cu- L_x / Å	1.97 (0.018)	1.99 (0.02)	1.91 (0.11)
$\sigma^2(L_x)$ / Å ²	0.0009 (0.0009)	0.0009 (0.0009)	0.0069 (0.0176)
Cu- L_y / Å	2.71 (0.03)	2.71 (0.03)	2.05 (0.07)
$\sigma^2(L_y)$ / Å ²	0.0036 (0.0032)	0.0039 (0.0034)	0.0153 (0.0153)
Cu- L_z / Å	1.88 (0.02)	1.90 (0.02)	1.96 (0.06)
$\sigma^2(L_z)$ / Å ²	0.0035 (0.0033)	0.0033 (0.0031)	0.0025 (0.0021)
E_0	0.39 (0.91)	0.34 (0.90)	-2.81 (3.78)
S_0^2	0.88 (0.12)	0.92 (0.13)	1.00 (0.09)
χ^2	4.75	4.37	7.99
R / %	21.1	20.3	27.46

Figures for these fittings are shown 7-9.

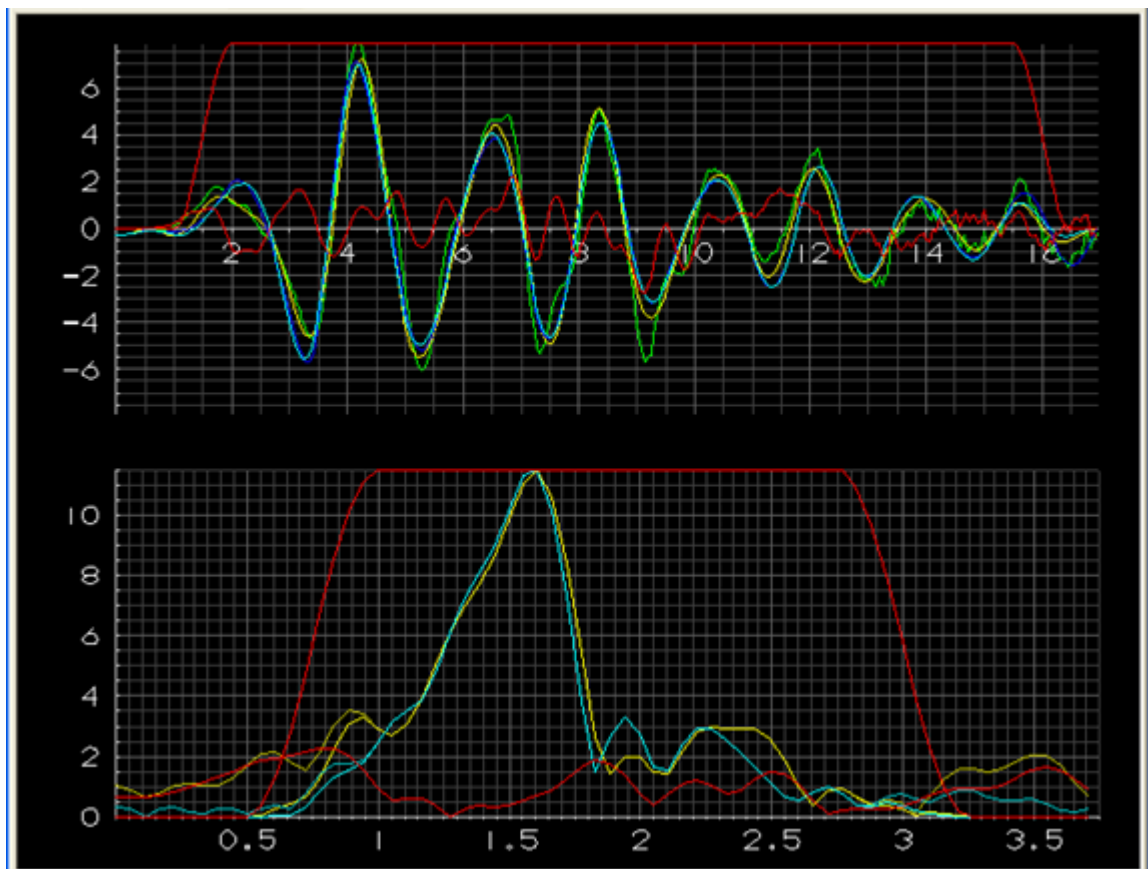


Fig.7: Fitting when all the atoms are oxygen around the copper atom

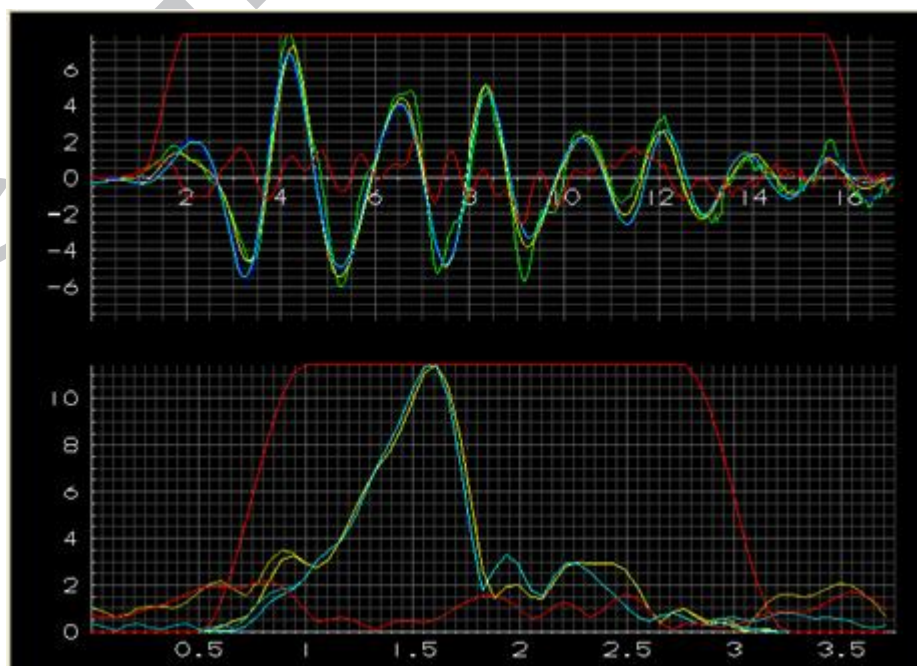
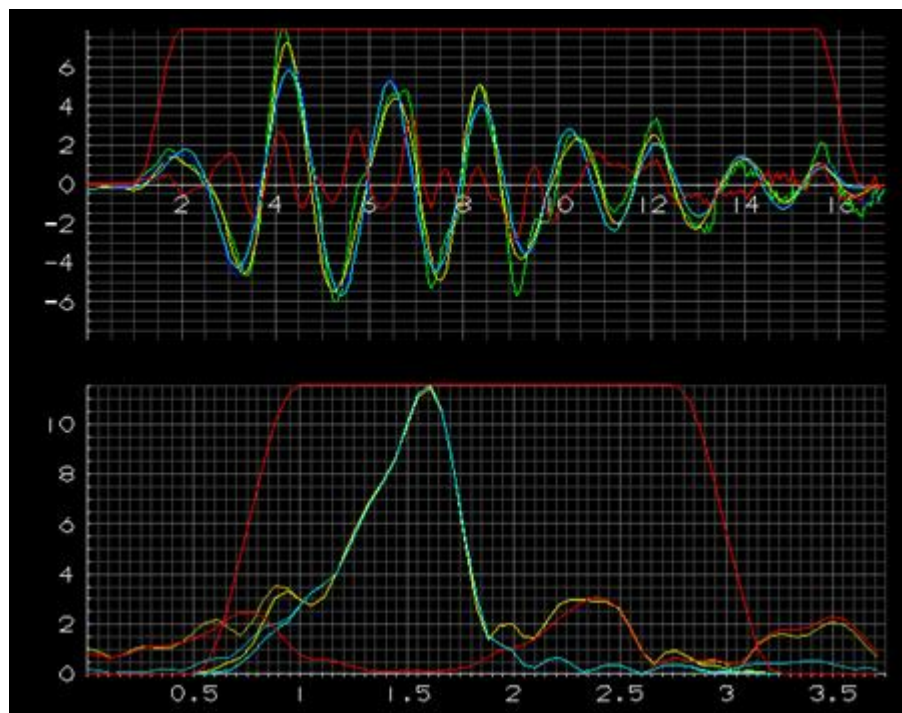


Fig. 8: Fitting when four atoms are oxygen and two are nitrogen around copper**Fig. 9:** Fitting when four atoms are oxygen and two are sulfur around copper

It is clear from all the above data and figures that the simplicity of the model fails to account for longer range scattering interactions with atoms in the second coordination sphere. This will show up in the longer R range and as higher frequency oscillations in the XAFS. While an extension of the model to include those interactions these additions would increase the number of parameters required to fit the data. Both in terms of the quality of the data and the questions under consideration. The extension of the analysis to include those factors is not justified.

Hence there are high frequency oscillations in the residuals and these indicate longer range scattering interactions not included in the model. This is not at all surprising and is likely to relate to the backbone of the amino acids.

Conclusion of XAFS analysis

The XAFS analysis strongly suggest a significant axial distortion. While it is likely that the coordination of the amino acid is through O and N atoms there is little difference between the statistics of the 4O and 2O, 2N refinements. This is not surprising given the closeness of the atomic numbers of these atoms. Hence coordination through the oxygens of carboxylate and oxygen of sulfoxide group is predicted in this complex. These modes of coordination are also predicted by FTIR spectra of these complexes.

3.8 Bioactivity

The complexes were dissolved in distilled water/DMSO to prepare 0.1% W/V solution. Antimicrobial activity was assessed by employing *Staphylococcus aureus*, *Escherichia coli* and *Sacchaomyces cerevisiae* as representative of Gram positive, Gram negative and eukaryotic microorganisms respectively.

A given solution (10 μ l) was dispensed on autoclaved Whatman filter paper No.1 discs of 6mm diameter. Growth of overnight incubated culture of a given test microbe in nutrient broth (10 μ l) was spread over solidified nutrient agar plate containing (15ml) of medium with the help of sterilized glass spreader. Five minutes were allowed the surface of the inoculated nutrient agar to absorb the fluid of inoculum before applying the discs. Then the loaded discs were picked up with the help of sterilized forceps and placed at least 15mm apart from the edge of the inoculated nutrient agar plates and 2cm apart from each other along with control discs loaded with 10 μ l of respective solvent. Each disc was then gently pressed with the help of blind end of a sterilized steel probe. Plates were then incubated for 18-24hrs at 37°C. After incubation, growth inhibition zones were measured in millimeters. Photograph of respective zones of growth inhibition were taken with the help of digital camera.

3.8.1 Results

Growth inhibition effects of Ethiin and its metal complexes are listed in Table 3.7. Ethiin alone did not show any bioactivity whereas the tested complexes exerted antimicrobial effects against the test organisms. However different compounds have different levels of antimicrobial activity against gram positive, gram negative bacteria and yeast. The results suggest that while being absorbed in the microbe cell, the complex making ability of ethiin have led ethiin to take with it, the metal ion, inside the microbe cell which, disturbed the ionic environment of cytoplasm and hence inhibited the growth of the microbe. Another mechanism can be the interaction of the complex with the DNA of the microbe resulting in its death. Such type of induction of antimicrobial activity in a ligand and its mechanism after complexation with metals has also been reported in literature[36-38]. The complexes reported here can be used as broad spectrum antibiotic compounds.

Table 3.7
Antimicrobial activity of all other complexes

Sr. No.	Compound	Test Organism		
		E. coli (mm)	S. aureus (mm)	S. cerevisiae (mm)
1	H ₂ O	R	R	R
2	DMSO	8	R	R
3	Dioxane:water 1:1	R	R	R
4	S-ethyl-L-cysteine sulfoxide	R	R	R
5	Bis(S-ethyl-L-cysteine sulfoxidato)Co(II)	12	9	14
6	Bis(S-ethyl-L-cysteine sulfoxidato)Ni(II)	12	10	16

Disc. Diameter = 6.00mm. R= The microbe was resistant to the applied substance.

4. Conclusion

Based on the spectral and physical data which indicates bidentate symmetrical carboxylate group coordination and sulfoxide coordination through oxygen atom in all the complexes, following octahedral structure have been proposed for the complexes of S-ethyl-L-cysteine sulfoxide shown in Fig. 10. The water solubility of these complexes also indicates their monomeric nature.

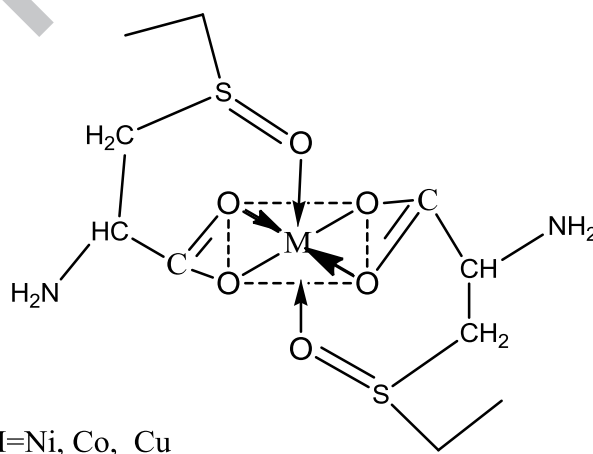


Fig. 10: Proposed Structure for the complexes S-ethyl-L-cysteine sulfoxide.

Such complexes with symmetric bidentate coordination of carboxylate group have already been reported for amino acids and acetate complexes [31,33,34]. Ethiin did not show any bioactivity but on complexation, antimicrobial effect is produced.

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ACCEPTED MANUSCRIPT

Appendix

Input file used for fit for Bis(S-propyl-L-cysteinato)Cu(II): $\text{Cu}(\text{C}_6\text{H}_{12}\text{NO}_2\text{S})_2$

File generated by the XFIT SAVE command

theory FEFF601

LEGS = 4

RMAX = 4

PW = 2

CW = 3

E = 1.00e+09

PH = 0

E0(0) = 0

E0i(0) = 0

S02(0) = 0.75

SHELL(0) = Cu

POT(0) = 0

N(0) = 1

x(0) = 0

y(0) = 0

z(0) = 0

sigma2(0) = 0

E0(1) = 0

E0i(1) = 0

S02(1) = 0

$$\text{SHELL}(1) = 0$$

$$\text{POT}(1) = 1$$

$$\text{N}(1) = 1$$

$$x(1) = 2.00$$

$$y(1) = 0$$

$$z(1) = 0$$

$$\text{sigma}2(1) = 0.002$$

$$E0(2) = 0$$

$$E0i(2) = 0$$

$$S02(2) = 0$$

$$\text{SHELL}(2) = 0$$

$$\text{POT}(2) = 1$$

$$\text{N}(2) = 1$$

$$x(2) = -2.04$$

$$y(2) = 0$$

$$z(2) = 0$$

$$\text{sigma}2(2) = 0.002$$

$$E0(3) = 0$$

$$E0i(3) = 0$$

$$S02(3) = 0$$

$$\text{SHELL}(3) = 0$$

$$\text{POT}(3) = 2$$

$$\text{N}(3) = 1$$

$$x(3) = 0$$

$$y(3) = 2.6$$

$$z(3) = 0$$

$$\text{sigma}2(3) = 0.004$$

$$E0(4) = 0$$

$$E0i(4) = 0$$

$$S02(4) = 0$$

$$SHELL(4) = 0$$

$$POT(4) = 2$$

$$N(4) = 1$$

$$x(4) = 0$$

$$y(4) = -2.6$$

$$z(4) = 0$$

$$\text{sigma}2(4) = 0.002$$

$$E0(5) = 0$$

$$E0i(5) = 0$$

$$S02(5) = 0$$

$$SHELL(5) = 0$$

$$POT(5) = 3$$

$$N(5) = 1$$

$$x(5) = 0$$

$$y(5) = 0$$

$$z(5) = 1.90$$

$$\text{sigma}2(5) = 0.002$$

$$E0(6) = 0$$

$$E0i(6) = 0$$

$$S02(6) = 0$$

$$SHELL(6) = 0$$

$$POT(6) = 3$$

$$N(6) = 1$$

$$x(6) = 0$$

$$y(6) = 0$$

$$z(6) = -2.06$$

$$\text{sigma2}(6) = 0.002$$

$$\text{absorber}[0] = \text{Cu}$$

$$\text{edge}[0] = \text{K}$$

$$\text{E0}[0] = 8992$$

$$\text{polarisation0}[0] = [0 , 0 , 0]$$

$$\text{normal0}[0] = [0 , 0 , 0]$$

$$\text{ellipticity0}[0] = 0$$

$$\text{O0}[0] = 1$$

$$\text{w}[0] = 1.00000$$

$$\text{kw}[0] = 3.00000$$

$$\text{N}[0] = 1024$$

$$\text{rmax}[0] = 28.35926$$

$$\text{k0}[0] = 2.00000$$

$$\text{k1}[0] = 14.25000$$

$$\text{kw0}[0] = 0.50000$$

$$\text{kw1}[0] = 0.50000$$

$$\text{ke0}[0] = \text{cosine}$$

$$\text{ke1}[0] = \text{cosine}$$

$$\text{r0}[0] = 1.00000$$

$$\text{r1}[0] = 2.75000$$

$$\text{rw0}[0] = 0.25000$$

$$\text{rw1}[0] = 0.25000$$

$$\text{re0}[0] = \text{cosine}$$

$$\text{re1}[0] = \text{cosine}$$

$$E0(0) > -15 \{1\}$$

$$E0(0) < 0 \{1\}$$

$$S02(0) > 0.5 \{0.1\}$$

$$S02(0) < 1 \{0.1\}$$

$$s1 < 0.02 \{0.01\}$$

$$s3 < 0.02 \{0.01\}$$

$$s5 < 0.02 \{0.01\}$$

$$s1 > 0.001 \{0.001\}$$

$$s3 > 0.001 \{0.001\}$$

$$s5 > 0.001 \{0.001\}$$

$$S1 == S2$$

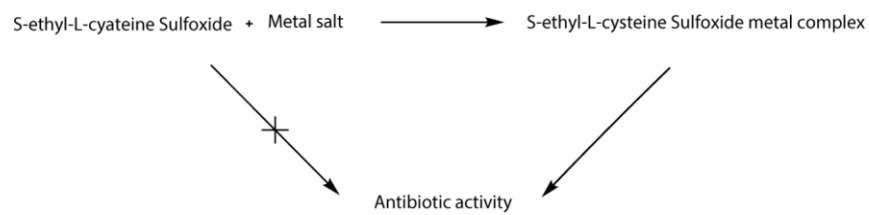
$$S3 == S4$$

$$S5 == S6$$

$$x2 == -X1$$

$$y4 == -y3$$

$$z6 == -z5$$



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