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Synthesis and Structural Analysis of Copper (II) Glutathione Complexes via Cu-S Linkage

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Abstract: This paper described the mechanochemical (solvent free) synthesis and structural analysis of stable copper (II) glutathione complexes. The bluish green copper (II) glutathione complexes were synthesized in mole ratios of 1:2 and 1:4 [(M: L) Metal: Ligand]. Infrared spectroscopy confirmed that copper (II) binding occurred via -SH of thiol. Microanalysis data agrees with the ML_2 in case of 1:2 and ML_4 in case of 1:4. Electronic spectra showed different transition in complexes. Powder X-ray diffraction data confirmed that phase changes were taken place in complexes with respect to the starting materials and size of crystals.

Key words: Mechanochemical (solvent free) Synthesis · Cu (II) complexes · Glutathione · Microanalysis · FT-IR Spectroscopy · UV-Visible Spectroscopy · Powder X-Ray Diffraction

INTRODUCTION

Low molecular weight thiols and disulfide including glutathione, cysteine and homocysteine are critical cellular components that play numerous important roles in metabolism and homeostasis. Glutathione is most abundant non-protein thiols has many wide-ranging functions within the cell including detoxification of free radicals and peroxides, regulation of cell growth and protein function and maintained the immune function [1]. It exists predominately in the reduced form Glutathione (GSH) at concentrations of 0.1-10 mM and is readily oxidized to the disulfide Glutathione (GSSG) [2]. Among glutathione role are to protect cells from reactive oxygen intermediates, UV radiation and heavy metal toxicity [3]. In the latter case, glutathione scavenges and sequesters heavy metal ions by coordinating them through its sulfydryl, there by inhibiting their binding to proteins and nucleic acids [4, 5-8]. Coordination of glutathione may also facilitate transfer to metal binding proteins, such as metallothionein. In some cases, glutathione reduces metal ions, such as Pt (IV) anticancer drugs, to species that coordinate or otherwise react with DNA [9-11]. A further role for glutathione has been suggested in intracellular copper transport and detoxification. In fact through the cysteine thiol group it can bind Cu^{2+} contributing to copper delivery to the appropriate of copper enzymes [12, 13]. The copper (I)/ (II)-glutathione systems are of particular importance for bioinorganic chemistry. Interaction of metals with glutathione metabolism is an integral part of the toxic response of many metals. Glutathione forms complexes with several heavy metal ions and thus functions in the protection of cells against metal toxicity.

As the predominant non-protein sulfhydryl in cell, glutathione plays several important roles. It has long been established that the thiols moiety of glutathione is important in antioxidant defense xenobiotic and eicosanoid metabolism and regulation of the cell cycle and gene expression (for review see) [14-18]. Although glutathione does not react directly with hydorperoxide, it is used as a substrate for glutathione peroxidase (GSHPx) [19]. The coordination chemistry of glutathione is of vital importance as it serves as a model system for the binding of metal ions by large peptide and protein molecules [20]. In this paper we described the mechanochemical (solvent free) synthesis and characterization of copper (II) complexes of glutathione with mole ratio 1:2 and 1:4.

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Experimental: All the chemicals and metal salt used in the synthesis were of reagent grade and used without further purification.

Synthesis of Cu (Ii)-glutathione Complexes

Experiment No.1: Appropriate drug L-glutathione (Sigma Aldrich) 6.1466 g (0.02 moles) and copper acetate monohydrate (Riedel-de Ha¸n) 1.9965 g (0.01 mole), were ground by use of an agate pestle and mortar. On grinding the drug L-glutathione, reduced with copper acetate monohydrate by solvent free method, acetic acid was released which was identified by the vinegar like smell. Reaction was completed after 3-4 hours. Completion of reaction was ascertained by cessation of acidic acid fumes and microcrystalline powder of sea green was obtained. The complex was characterized as such and after washing with methanol.

Experiment No. 2: The experiment was repeated (as mention above) by taking appropriate drug L-glutathione (Sigma Aldrich) 12.2932 g (0.04 moles) and copper acetate monohydrate (Riedel-de Ha¸n) 1.9965 g (0.01 mole). Microcrystalline powder of bluish green was obtained. The complex was characterized as such and after washing with methanol.

Microanalysis: Microanalysis for carbon, hydrogen, sulfur and nitrogen was carried out by VarioMICRO CHNS analyzer. An accurate weighed amount of sample was put in tin foil along with appropriate amount of V_2O_5 as an oxidizing agent. The sample was loaded on the microanalyzer and measurements were recorded.

FT-IR Spectroscopy: FT-IR spectra of the samples were obtained using IR-Prestige (Shamdzu, Japan) spectrophotometer in the range of 400-4000 cm^{-1}. The spectra were recorded by KBr disc method.

UV-Visible Spectroscopy: UV-Visible spectra of the samples were recorded by employing 2300 UV-Visible TECHCOMP (Shamdzu, Japan) dual beam spectrophotometer at room temperature in the range of 190-900 nm with the scanning speed of 1200 nm/min. The lower cut-off wavelength was found to be 215 nm.

Powder X-Ray Diffraction (PXRD): Powder X-Ray Diffraction (PXRD) spectra of the complexes were recorded on Bruker D8 advance, CuKα radiation. The diffraction spectra of samples were collected with a CuKα source (λ=1.540598 nm) and using a θ-2θ geometry, with a scanning time of 0.5 s and step of 0.03°.

Solubility of Complexes: Solubility of the complexes were determined in hot and cold water, *N, N*-dimethyl formamide (DMF), Dimethyl sulfoxide (DMSO) and other common organic solvents by shaking a small amount of complex in the solvent in a test tube.

RESULT AND DISCUSSION

The mechanochemical synthesis of the Cu (II)–GSH complexes resulted in the production of bluish green and light green powder which was sparingly soluble in DMF, DMSO and insoluble in all other aqueous and non-aqueous solvents. The solubility data of the complexes under investigation is given in Table 1.

To confirm whether the material synthesized were surly the above name complexes. Carbon, Nitrogen, Hydrogen and Sulfur analysis carried out. The experimental and Calculated values agreed with each other but all value do not fit accurately in the proposed composition due to the fact that some unreacted materials may be present in the product, but it gives a fairly good idea that it matches with Cu (II)-GSH with 1:2 and 1:4 ratios and data is represented in Table 2.

Table 1: Solubility data of Complexes

	Solvents					
Complexes	Water	Methanol	DMSO	DMF	Pyridine	Acetone
Cu-GSH 1:2	IS	IS	IS	SP	SP	IS
Cu-GSH 1:4	IS	IS	IS	SP	SP	IS

Key: IS: Insoluble; SP: Sparingly Soluble

Table 2: Microanalytical Data [% FOUND (CALCULATED)]

Complexes	Color	% N	% C	% H	%S
Cu-$[GSH]_2$	Bluish Green	12.38 (12.41)	35.55 (35.63)	4.77 (4.76)	9.50 (9.53)
Cu-$[GSH]_4$	Light Green	12.99 (13.05)	37.40 (37.33)	5.12 (5.18)	9.37 (9.34)

Abbreviation

GSH = L-Glutathione Reduced

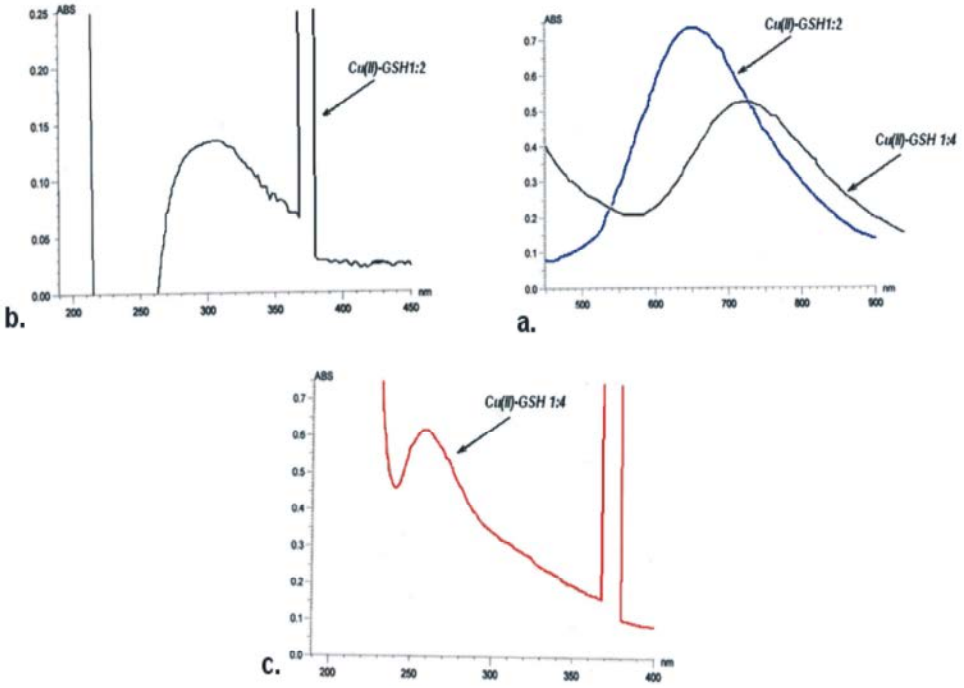

Fig. 1: Electronic Spectra of Cu (II)-GSH complexes in 1: 2 and 1:2 mole ratios. (*a*) In the range of 900-500 nm (*b*) In the range 200-450 nm (c) In the range of 400-200 nm

Table 3: UV-Vis data of Complexes:

	λmax (nm)	
Complexes	Visible range	UV range
Cu-GSH 1:2	648nm	306nm
Cu-GSH 1:4	702nm	262nm

The UV-Vis measurement of Cu (II)-Glutathione complexes were carried out in DMF solution (green color solution produced). The absorption band assignment is listed in Table 3 and spectra are shown in Fig. 1. The transition occurring in Cu-GSH complexes are of two types. One confined to the d-orbital of the Cu atom (ligand field band) and other is incipient charge transfer (LMCT or MLCT) [21]. The band observed at λ_{max} 648 nm and 702 nm in visible region is due to the d-d electronic transition of Cu metal ion, since it is a d^9 system. It has been suggested that visible spectrum of a d^9 system in an octahedral field is rare due to the John-Teller effect. In actual practice, a non-symmetric absorption band result due to the John-Teller effect. Furthermore, the ground state in Cu^{2+} (d^9) is doubly generated and the excited state is triply generated, hence board spectrum result. One the other hand the ligand L-glutathione has lone pair on N atom and π electron in carbonyl group so it shows intense absorption band (λ_{max}) at 306 nm and 262 nm resulting n-σ^* and n-π^* electronic transition respectively.

Cu (II)-GSH complexes were analyzed using infrared spectroscopy. The FT-IR spectral data of metal complexes and reactants are given in Fig.2 and assignments are given in Table 4. In FT-IR spectra of the metal complexes all the bands due to the ligand were present in addition to some new bands. The result indicates that the L-Glutathione and complexes show strong and board absorption for -OH, H_2O and -NH stretching in the region of 3340-3240 cm^{-1}. -CH absorption bands are obtained at 3050-3010 cm^{-1}. The absorption band at 2578 cm^{-1} due to the *ν*(SH), which is disappear in the Cu(II)-GSH complexes in 1:2 and 1:4 ratio indicates the deprotonation of the thiol group and subsequent binding of Cu (II) to glutathione via the sulfur atom [22]. The band at 1602 cm^{-1} is assigned due to –NH stretch in complexes and ligands. The sharp band at 1400 cm^{-1} in the ligand and 1409 and 1411 cm^{-1} in the complexes are assigned due to the *ν*CO have been indicating no co-ordination with Cu atom through the COO^- group of ligand. The strong band located at 750-690 cm^{-1} is still due to the C-S stretching vibration of complexes. The intensity enhancement of this band indicates that important electronic arrangement might occur in the S atom level. This fact implies that S atom is directly involved in the ligand to copper metal [23]. Furthermore, the FT-IR curve in the finger print region (below 1300 cm^{-1})

Table 4: Infrared bands, assignments of L-Glutathione reduced and Copper (II) Glutathione Complexes in mole ratio of 1:2 and 1:4.

L-Glutathione	Cu-GSH 1:2	Cu-GSH 1:4	Assignments
3338.78	3338.78	3336.9	-NH Stretch
3244.27	3250.05	3240.41	-OH Stretch
3024.38	3028.24	3026.31	-CH Stretch
2578.33	Absent	Absent	-SH Stretch
1977.04	1975.11	1971.25	-CH Out of Plane
1712.79	1712.79	1712.79	-C=O Stretching
1602.85	1606.70	1597.06	$-CO_2^-$ Stretching
			-NH Stretch
1539.20	1539.20	1537.27	$-NH_3^+$ Deformation
1400.32	1409.96	1411.89	-CO Stretching
			$-CH_2$-CO Stretch
1336.67	1334.74	1336.67	-OH Deformation
1259.52	1263.37	1265.30	-OH Deformation
929.69	929.69	929.69	-OH Deformation
819.75	821.68	821.68	-CN Stretch
748.38	763.81	761.88	-CH Out of Plane (4 Hydrogen)

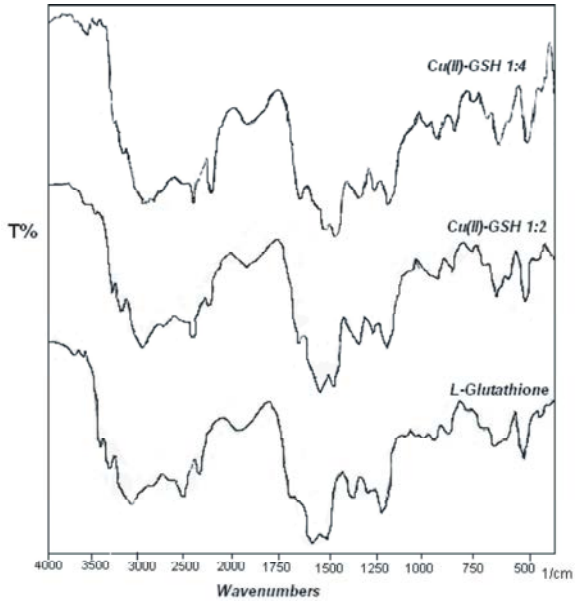

Fig. 2: IR spectra o fL-Glutathione and the Cu(II)-GSH complexes in 1:2 and 1:4 mole raios of Cu (II): glutathione

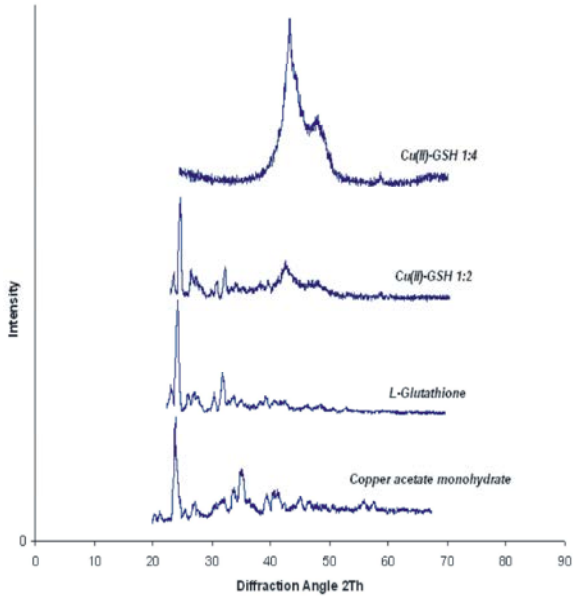

Fig. 3: PXRD Spectra of Reactant and Complex

confirms that the Cu (II)-glutathione complexes in 1:2 and 1:4[Cu (II)-GSH] are different form the originating parent molecules, as they posses different spectroscopic signals.

The PXRD spectra of the reactants and products are shown in Fig. 3 and data are given in Table 5. It can be seen that the PXRD pattern of the products is substantially different from those of the reactants. One new peak observe through a range of 2θ angles 40-45° showed lower intensity in case of Cu(II)-GSH 1:2 but higher intensity in case of Cu(II)-GSH 1:4. The major peaks [Pos. (2theta = 22.5962°)] for glutathione reduced in Cu(II)-GSH 1:2 and disappeared in Cu (II)-GSH 1:4 complexes which suggests the formation of the new phase due to complexations.

The prominent well resolved Bragg's peak at specific 2θ angle reveals the high crystalline nature of the crystals. The crystallite sizes (D) were calculated using Scherer's formula from the full width at half maximum (FWHM)

$$D = k\lambda / \beta \cos\theta$$

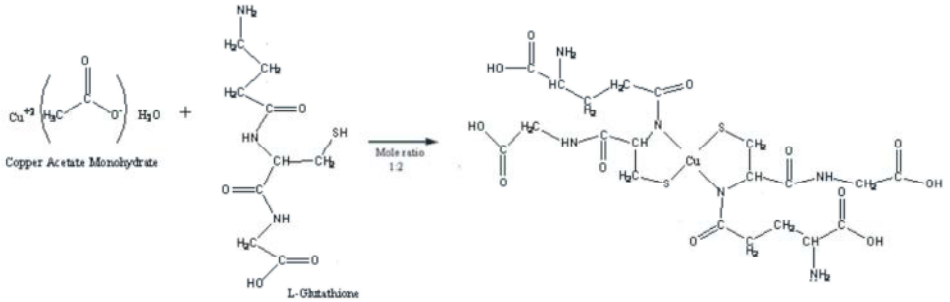

Fig. 4: Proposed Strcturs of Cu (II)-GSH 1:2 and 1:4

Table 5. X-Ray Powder Diffraction Data of Cu-GSH 1:2 and 1:4 mole ratio

Cu-GSH 1:2			Cu-GSH 1:4		
2θ	d-spacing [A]	FWHM	2θ	d-spacing [A]	FWHM
24.5251	3.62978	0.0886	24.7489	3.59747	0.4723
30.1436	2.96480	0.2362	33.8630	2.64719	0.2952
33.1925	2.69912	0.3542	44.5043	2.03684	0.5090
44.3819	2.02172	0.7085	50.8050	1.79716	0.3542
50.6698	1.80164	0.0886	52.1670	1.75340	0.3542
51.6175	1.77077	0.5904	64.8108	1.43857	0.1181
63.6381	1.46235	0.2952	69.0014	1.35995	0.1080
65.3626	1.42775	0.2952	69.4427	1.35351	0.1181
67.1090	1.39478	0.2925	72.9998	1.29501	0.0720
69.1029	1.35820	0.1800	75.6180	1.25654	0.3600

Where, β>the broadening of diffraction line measured at half of its maximum intensity, λ>X-ray wavelength (1.5406Å), θ>Bragg's angle, k>constant (0.9). The calculated average crystallite size is about 101 nm in 1:2 mole ratios and 203 nm in 1:4 mole ratios.

Based on Spectroscopic data, elemental analysis CHNS and powder X-ray Diffraction enable us to predict possible structure as shown in Fig. 4.

CONCLUSION

Cu (II)-Glutathione complexes in mole ratio 1:2 and 1:4 (Cu-Glutathione) have been synthesized by mechanochemical method at room temperature. Chemical composition of the synthesized complexes was established by CHNS analysis. The FT-IR analysis verified all the functional groups and binding occurred almost exclusively through bridging sulfur bonds on the thiols of glutathione. UV-Visible spectral verified electronic transition in complexes. Different between the phase changes of reactant and complexes and size of crystals were determined by Powder X-ray diffraction.

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