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Solvent Free Synthesis of Copper(II) Cysteine Complexes

[1]Manan Ahmed, [1,2]Muhammad Saeed Iqbal, [1]Naveed Tahir and [1,3]Amjad Islam

[1]Department of Chemistry, GC University, Katchery Road, Lahore-54000, Pakistan
[2]Department of Chemistry, FC College (A Chartered University), Ferozepur Road, Lahore-54600, Pakistan
[3]Industrial Chemicals Division, Guides Corporation, Davis Road, Lahore-54000, Pakistan

Abstract: This paper describes the solvent free synthesis and structural analysis of stable copper(II)-cysteine complexes. The sky blue Cu(II)-cysteine complexes were synthesized in mole ratios of 1:2 and 1:4 [(M: L) Metal: Ligand]. Infrared spectroscopy confirmed that Cu(II) binding occurred via -SH of thiol. Microanalysis data agreed with the ML_2 in case of 1:2 and ML_4 in case of 1:4. Powder x-ray diffraction data confirmed that phase changes had taken place in complexes with respect to the starting material. Computed structures were analyzed and compared with the experimental data.

Key words: Solvent Free Method · Copper(II)-Cysteine Complexes · Powder X-Ray Diffraction· FT-IR Spectroscopy

INTRODUCTION

The transition or *d*-block metal ions manganese, iron, cobalt, nickel, copper, zinc and in more specialized case, molybdenum, tungsten and vanadium, have been shown to be important for biological systems. These metal ions are abundantly found in nature and exclusively found as constituents of proteins [1]. Copper is essential for life being the co- factor of enzyme that involves in processes such as respiration (cytochrome dismutase). However, copper ions easily cycle between Cu(I) and Cu(II), some copper complexes could take part in non-enzymatic redox processes. In some cases, these processes alter the cell status by acting on intercellular redox potential. In particular in the presence of molecular oxygen, copper ions catalyze oxidation of biomolecules and produce reactive oxygen species (ROS) [2], which are being responsible for molecular damage (protein or DNA degradation, membrane per oxidation etc) and alter signal transduction cascades [3]. Sulfur and aromatic nitrogen-based anions form relatively stable compounds with Cu(I), due to favorable soft-acid base interaction. Cu(I) thiolate which regarded as being polymeric have been directly prepared by the addition of an excess of thiols to aqueous solution of Cu(II) salts [4, 5] or by electrochemical reduction of Cu(II) in the presence of thiols [6].

Cysteine is involved in biological reductive/oxidative chemistry (via disulfide formation) and it can participate in radical reactions [7, 8]. It can also participate in regulator functions i.e., signaling through several stable post translational modification with proteins including formation of nitrosothiols (RS-NO), sulfenic acid (S-OH) and glutathione disulfide [9-13] . Metal-cysteine complexes perform a variety of functions in biological environment and medical fields. The Cu(II) complexes thus prepared are expected to possess high cytotoxicities which as such may be considered as potential anti-tumor agents [14-15] and it has been shown that the combination of cysteine, glutathione and copper can inactivate HIV protease [16, 17].

Most studies regarding the copper cysteine complexes discuss their production in aqueous solution [14, 18, 19]. When copper(II) salts react with cysteine in aqueous solution, a redox reaction takes place. In this case, Cu(II) is reduced into Cu(I) and thiols are oxidized which may result in disulfide bond. Whereas Cu(I) interacts with thiols, thus a stable complex formation takes place due to the favorable soft-acid base interaction. Masoud *et al.*, [14] described the synthesis of a pale green Cu(II)-cysteine complex in distilled water with a stoichiometry ratio of 1:1.2, Cu(II)-cysteine complex: water and Dokkan *et al.* [15] described the synthesis of Cu(II)-cysteine complex in ethanol using mole ratios of 1:2, 1:4 and 1:6, Cu(II): cysteine. Their findings suggested that the binding of copper occurs through carboxylate and thiol group. However, based on their data it was suggested that they formed Cu(I)-cysteine or impure Cu(II)-cysteine complex similar as reported by Gale *et al,* [20].

Corresponding Author: Manan Ahmed, Department of Chemistry, GC University, Katchery Road, Lahore, 54000, Pakistan. Tel: +92-321-4723289.

The structure determination of metal-cysteine complexes is extremely important for understanding how cysteine-rich proteins, such as metallothioneins and phytochelatins, uptake and bind metals like copper. However, mostly Cu-cysteine complex formation is not available through chemical manufacturing.

Herein we report the synthesis of stable copper(II)-cysteine complexes by solvent free method using mole ratio 1:2 and 1:4 because these complexes are difficult to prepare in the presence of any solvent due to the redox reaction as the oxidation readily takes place in solution. In order to prepare Cu(II)-cysteine stable complexes, a solvent free method has to be tried.

EXPERIMENTAL

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

Experiment No.1: Appropriate drug L-cysteine (Sigma Aldrich) 2.423g (0.02M) and copper acetate monohydrate (Riedel-de Ha¸n) 1.9965g (0.01M) were ground by use of an agate mortar and pestle. On grinding the drug L-Cysteine with copper acetate monohydrate by solvent free method, acetic acid was released which was identified by the vinegar like smell. Reaction was completed after 4-5 hours. Completion of reaction was ascertained by cessation of acetic acid fumes. Microcrystalline powder of pure sky blue colour was obtained. The complex was characterized as such and after washing with methanol.

Experiment No.2: The experiment was repeated (as mentioned above) by taking appropriate drug L-cysteine (Sigma Aldrich) 4.8464g (0.04M) and copper acetate monohydrate (Riedel-de Ha¸n) 1.9965g (0.01M). Microcrystalline powder of pure light sky blue colour was obtained. The complexes were characterized as such and after washing with methanol.

Microanalysis: Microanalysis for carbon, hydrogen, sulfur and nitrogen were carried out by Vario MICRO CHNS analyzer. An accurately 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 (Shimadzu, Japan) spectrophotometer in the range of 4000-400 cm^{-1}. The spectra were recorded by KBr disc method.

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 used a θ-2θ geometry, with a scanning time of 0.5s and a step of 0.03°.

Solubility of Copper(II)-Cysteine 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.

RESULTS AND DISCUSSION

The solvent free synthesis of the Cu(II)-cys complexes resulted in the production of sky blue powder which was insoluble in dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) and all other aqueous and non-aqueous solvents. The solubility data of the complexes under investigation is given in Table 1.

CHNS data of the complexes is given in Table 2. To confirm whether the material synthesized was surly the above name complexes, carbon, nitrogen, hydrogen and sulfur analysis was carried out. The experimental and calculated values agree with each other but all values do not fit accurately in the proposed composition due to the fact that some untreated materials may be present in the product, but it gives a fairly good idea that it matches with Cu(II)-cys 1:2 and 1:4 ratios.

Cu(II)-cys complexes were analyzed using infrared spectroscopy. The FT-IR assignments of the complexes and reactant are shown in Table 3. In the FT-IR spectra of the complexes, all the bands due to the ligand were present in addition to some new bands. The absence of infrared peak between 450 cm^{-1} and 490 cm^{-1} which donates the disulfide bond, indicates that Cu(II) did not oxidize cysteine to form cystine. The disappearance of the thiol (-SH) stretch at 2551 cm^{-1} in the 1:2 and 1:4 complexes indicates the deprotonation of the thiol group and subsequent binding of Cu(II) to cysteine via the sulfur atom. The appearance of strong IR band for NH_2 stretch at 3169 cm^{-1} and disappearance of bands in complexes signifies their deprotonation. However the presence of other NH_3^+ bands at 1591 cm^{-1} and 1120 cm^{-1} present in both, free ligand and the Cu(II)-cysteine complex indicates only a partial deprotonation. Shifts in the asymmetric and symmetric stretches of carboxylate functionality (Table 3) due to coordination with Cu [21] were not obviously persent in the spectra of the

Table 1: Solubility data of complexes

	Solvents					
Complexes	Water	Methanol	DMSO	DMF	Pyridine	Acetone
Cu-Cys1:2	IS	IS	IS	IS	IS	IS
Cu-Cys1:4	IS	IS	IS	IS	IS	IS

IS: Insoluble

Table.2: Microanalytical data [% Found (Calculated)]

Complexes	N	C	H	S	Copper
Cu-Cys 1:2	9.32 (9.214)	25.49 (23.714)	4.371 (3.949)	22.44 (21.101)	25.34 (26.22)
Cu-Cys 1:4	9.82 (9.184)	26.69 (23.636)	5.003 (4.264)	23.708 (21.03)	11.03 (10.87)

Abbreviations Cys = l-Cysteine:

Table.3: Infrared band assignments of L-cysteine and copper(II)-cystenine complexes in mole ratios of 1:2 and 1:4

L-Cysteine	Cu-Cys 1:2	Cu-Cysl 1:4	Assignments
3647.39			-OH Stretch
	3595.31	3595.31	-OH Stretch
	3518.16	3514.30	-OH Stretch
	3448.72	3450.65	-NH Stretch
	3296.35	3294.42	-OH Stretch
	3228.84	3226.91	-OH Stretch
3169.04			-NH Stretch
	3043.67	3020.53	-CH Stretch
2993.88	2987.74		-CH Stretch
2551.82			-SH Stretch
2351.23	2353.16	2349.30	Not assigned
2081.19	2100.48	210241	-CH Stretch
1519.27	1625.99	1624.06	$-CH_2$ Stretch
			$-NH_3^+$ Deformation
	1492.90		-CH Deformation
1408.04	1400.32	1400.32	-CO Stretch
1344.38	1352.10	1361.74	-OH Deformation
1298.09			-OH Stretch
1195.87	1209.37	1209.37	-CH Stretch
1143.79	1120.64	1122.57	-CO Stretch
1062.78	1062.63		-CO Deformation
997.20	966.34	989.48	-CH Deformation
941.26			-OH Deformation
	918.18		-OH Deformation
866.04	848.68	856.39	-CH Deformation
815.89			-CH Stretch
692.44	667.37	665.44	Not assigned
638.44			Not assigned
538.14		547.78	Not assigned

Cu(II)-cys complexes. The symmetric stretches, in plane bending and out of plane bending frequencies (1408 cm^{-1}) of the carboxylate functionality remain relatively unchanged in the Cu(II)-cys complexes compared to the free ligand. A strong band of -CN at 1195 cm^{-1} present in L-cysteine and copper cysteine complexes indicates that -CN did not take any part in binding.

These data show that the carboxylate functionality plays no role in binding Cu to the cysteine. Further evidence of this can be observed as the CO stretch and in plane bending of the OH group associated with carboxylate functionality in the 1400 cm^{-1} and 1340 cm^{-1} region were similar in both free ligand and the Cu(II)-cys complexes. This evidence indicates that sulfur plays a major role in binding and carboxylate plays a minor role in binding. Furthermore, the FTIR curve in the finger print region (below 1300 cm^{-1}) confirms that the Cu(II)-cysteine 1:2 and 1:4 complexes are different from the originating parent molecules, as they posses different spectroscopic signals.

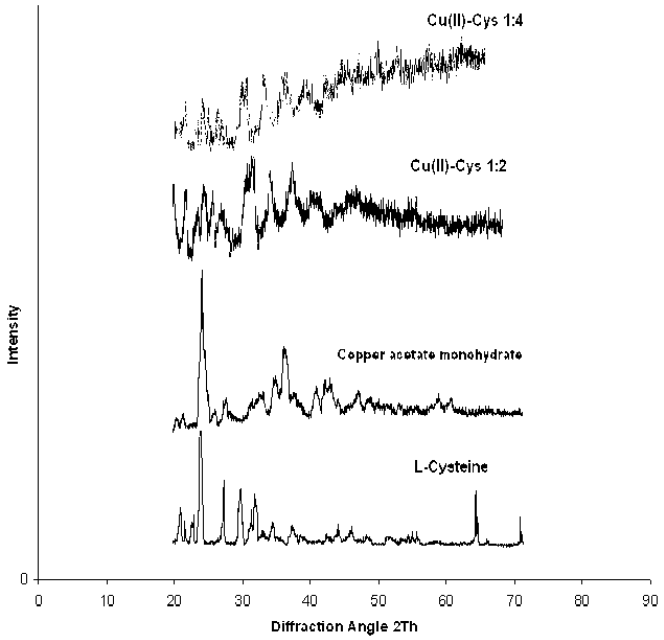

Fig. 1: Powder X-Ray diffraction spectra of raw matertials and complexes.

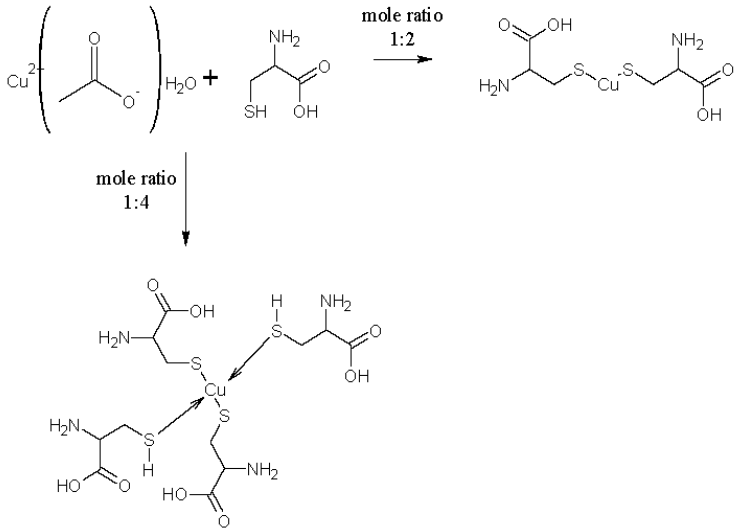

Fig. 2: Structures of copper(II)-cysteine complexes in mole ratios 1:2 and 1:4.

The powder XRD spectra of the reactants and products are shown in Fig. 1. The diffraction pattern shows that all of the copper cysteine in different mole ratio is substantially different from those of the reactants. The major peaks [Pos. (2Th = 24.6439)] for cysteine and copper acetate [Pos (2Th = 24.965)] reduce or disappear in Cu(II)-cysteine 1:2, 1:4 complexes which suggests the formation of the new phase due to complexation.

Based on these results, the likely structures of the complexes formed are proposed to be as shown in Fig. 2

CONCLUSION

Cu(II)-cysteine complexes in mole ratio 1:2 and 1:4 (Cu-cysteine) have been synthesized by solvent free method at room temperature. Chemical composition of the synthesized complexes was established by CHNS analysis. The FTIR analysis verified all the functional groups and binding occurred almost exclusively through bridging sulfur bonds on the thiol of cysteine. Differences between the phase changes of reactants and complexes were determined by powder x-ray diffraction.

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