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Antimicrobial Studies of Copper (II) Sesame Urea and Thiourea Complex Against *Staphylococus Aureus*

Asha Meena
Assistant Professor, Department of Chemistry, S. D. Govt. College Beawar, Ajmer, Rajasthan, India

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ABSTRACT

In the present work a systematic study give an overview of new findings in the field of biocidal activities for copper (II) sesame soap complex with urea ligand. The aim of this research work is to present fundamental chemical properties and new investigations of coordination compounds of some transition metal ions with an overview of medicinal applications. Transition metals appear in almost every facet of our day-to-day life, from industrial uses such as the manufacture of construction and building materials, tools, vehicles, up to cosmetics, paints and fertilizers. Their reactions are in general important in many technical processes such as catalysis, material synthesis, photochemistry, as well as, in biology and medicine. The present work deals with the study of synthesis and characterization of biological properties of novel copper (II) soap urea complex.

Keywords: Biocidal Activities, Thermogravimetric Analysis, Copper (II) Sesame Soap Complex, Degradation, Edible Oils.

I. INTRODUCTION

From the literature survey, it appears that copper (II) soap complexes with nitrogen and sulphur containing ligands have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities [1-2]. Research has shown significant progress in industrial utilization of these copper soap complexes as antimicrobial activities against *Staphylococus aureus*. These results shows that nature of different nitrogen and sulphur containing ligands coordinated with metal ion play a significant role in the inhibition activity [3-4]. In recent times the coordination chemistry of nitrogen and sulphur containing ligands has reputed greater importance in view of the fact that several of these compounds have been found to be biologically active and have found use in medicine as well as in industry [5,6,7]. Metal complexes of the ligands containing sulphur and nitrogen as donor atoms are known to obtain bactericidal, antiviral and carcinostatic activities [8-9]. Benzothiazoles are bicyclic ring system with various applications which have been the subject of great importance because of their biological activities. The aim of the present study to synthesis safer surfactants and to describe their degradation from environment.

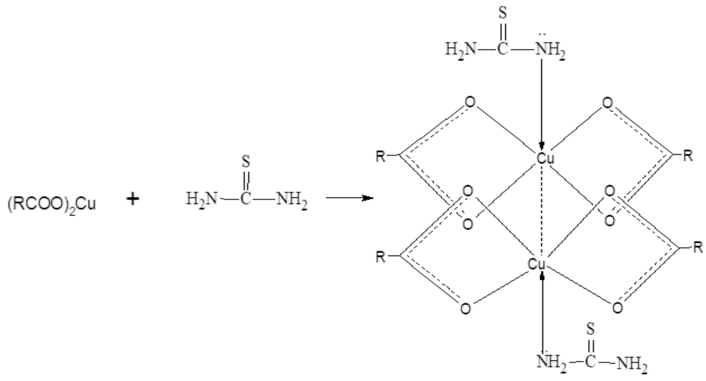
The present study focused on the thermogravimetric analysis of copper (II) soap complexes derived from edible oil such as sesame oil with ligand containing nitrogen, oxygen and sulphur atoms, which are crucial for their significant activity. In addition microbiological influence of the copper (II) soap complexes against *staphylococcus aureus* are investigated and reported. This review reveals that the pharmacologically interesting copper soap complexes could be a suitable strategy to develop novel therapeutic tools for the medical treatment [10-11].

II. METHODS AND MATERIAL

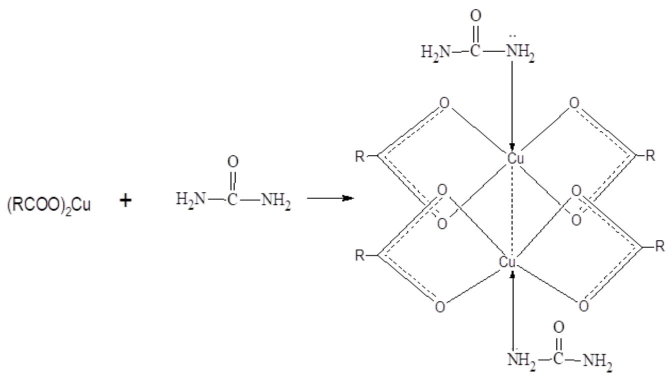
Copper (II) soaps were prepared by direct metathesis process. In this process, the oils were refluxed with 2N KOH solution and ethyl alcohol for about 3 hours. 1N HCl was used for neutralization of excess KOH. After adding required amount of saturated solution of $CuSo_4$ at 50-55 °C under vigorous stirring. The green precipitate thus obtained was filtered and then washed with hot distilled water and alcohol. After initial drying in air oven at 100-105 °C, these copper soaps were further purified by recrystallization with hot benzene several times. The metal was analyzed by standard procedure [12]. These copper soaps were of green in colour are obtained. Care was taken to avoid traces of water which were found to hydrolyze the soap.

Scheme-1: Synthesis of copper (II) soap (where R represents the mixture of various long chain segments as per composition of the oil).

Chemical reaction for synthesis of complexes



Scheme-2: Synthesis of copper (II) soap-thiourea complex.



Scheme-3: Synthesis of copper (II) soap-urea complex

The copper (II) soap complexes were prepared by reacting ethanolic solution of ligand (Urea and Thiourea) with copper (II) soap in 1:1 molar ratio. In 25-30 ml of ethyl alcohol, 0.001 moles of ligand molecule was dissolved and in 10-15 ml of benzene, 0.001 moles of copper (II) soap derived from sesame oil was dissolved and then ethanolic solution of ligand was added in it. After this reaction mixture was refluxed for about two hours with constant stirring. The solid precipitate which is separated on cooling was filtered, washed with hot distilled water and ethyl alcohol and dried in vaccum [13]. The dried complexes were then purified and recrystallized with hot benzene twice. These complexes are solids and dark green or greenish brown in colour, which are soluble in various solvents such as benzene, ether and methanol-benzene mixture but are insoluble in water, all the copper soap complexes are quite stable.

III. RESULTS AND DISCUSSION

IR spectral analysis

In order to study the structure of copper (II) sesame soap complex, the Infra-red spectra of compound was obtained on a spectrophotometer from Dept. of chemistry, S.P.C. Govt. College Ajmer in the range from 4000-400 cm^{-1} (Fig 1).

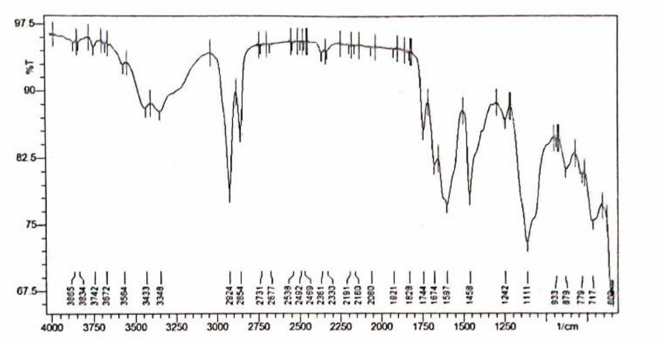


Figure 1. IR spectra of Copper (II) sesame thiourea complex

ESR spectral analysis

The ESR spectra of complex was recorded at liquid nitrogen temperature (LNT) from SAIF, IIT, POWAI, Mumbai using TCNE(g=2.00277) as a internal standard (Fig 2). The ESR spectra of Copper sesame soap complexes revealed three g values(g‖, g┴ and gav). The values of ESR parameters g‖, g┴ and gav are greater than the value of go i.e.2.0027. The obtained data indicates that the distortion from the regular octahedron has taken place in the shape of the complex. Furthermore, the trend g‖ > g┴ for the complex suggested that the unpaired electron is localised in dx2-y2 orbital. These factors indicates that complex possess elongated octahedral geometry and the values of g‖ are suggestive of covalent environment in metal-ligand bonding [14].

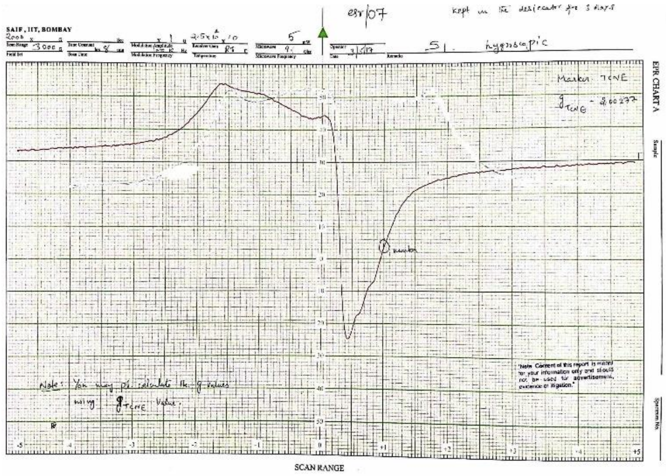


Figure 2. ESR spectra of Copper (II) sesame urea complex

NMR spectral analysis

[1]H NMR Spectra was recorded at CDRI, SAIF, Lucknow using C_6D_6 as reference.

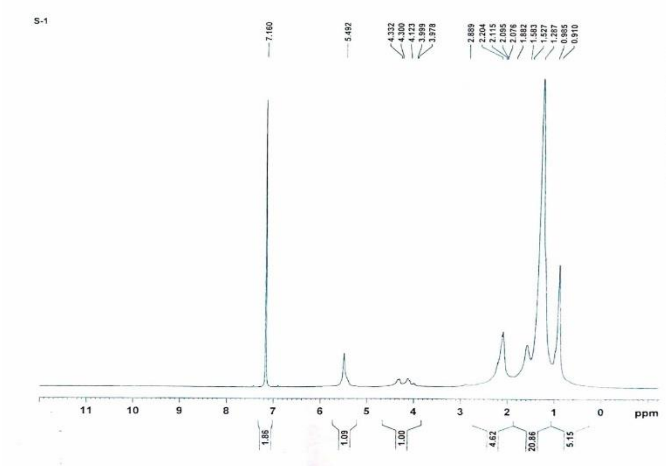


Figure 3. NMR spectra of Copper (II) sesame urea complex

All the above peaks (Fig 3) are due to the long chain fatty acid content (R) of the soap molecule [(R–$COO)_2Cu$]. Vinylic proton exhibits a signal around 5.492-5.500 δ. The ligand moiety of the complex molecule is very much distinguished with the peaks of aromatic NH_2 protons between 3.2-4.2 δ.

Antimicrobial Studies

All the Copper (II) sesame soap complexes were screened for their antibacterial activity against *Staphylococcus aureus*. These complexes were tested at different concentrations after 24 and 48 hours incubation times and zone of inhibition have been measured in mm. The complexes were dissolved in hot benzene [15,16]. Stock solutions of tested compounds were prepared in benzene to a final concentration of 50mg/ml. Two different dilutions C_1 (50mg/ml) and C_2 (25mg/ml) were made to get antimicrobial activity of each compound against *Staphylococcus aureus*. Dilutions were made in benzene. Streptomycin was used as positive control (300mcg/ml concentration).

Mueller-Hinton agar medium was used for antimicrobial activity of complexes on two different concentrations by disc/ well diffusion susceptibility testing. Fresh cultures of *Staphylococcus aureus* strain ATCC-25923 were inoculated in peptone water and kept for incubation for 30 minutes at 37 °C. Inoculum size of bacteria was adjusted using McFarland turbidity standard as reference [17]. The bacterial suspensions were compared to 0.5 McFarland turbidity standard. Microbial culture was swabbed onto the Mueller-Hinton agar surface through sterile cotton swab sticks. After proper marking of plates, 50μl extracts from different dilutions prepared was loaded into the respective wells. The swabbed *Staphylococcus aureus* plates were kept for incubation at 37 °C for 24-48 hours [18].

Table 1 and 2 shows the biological activities of copper (II) soap complexes determined by screening against bacteria at 5×10^4 ppm and 2.5×10^4 ppm. The copper (II) sesame soap complexes with ligand like urea and thiourea were screened for their antibacterial activity against *Staphylococcus aureus.* These compounds were tested at different concentrations and zone of inhibition have been measured in mm [19,20]. The antibacterial activity results, presented in Tables 1 and 2 respectively. The enhanced activity of newly bio-based copper (II) soap complexes can be explained on the basis of chelate formation, the presence of donor atoms such as nitrogen, sulphur and the structural compatibility with molecular nature of the toxic moiety [21-23]. In the complex, polarity of central metal ion reduces mainly due to partially sharing of its positive charge with the donor ligands and π- electron delocalization over the whole chelate ring. Such chelation could enhance the lipophilic character of the central metal atom, which consequently helps the penetration of the bacterial cell membranes and restricts further growth of the micro-organisms. The efficiency of antimicrobial activity of copper (II) soap complexes reveals that these complexes are more powerful antimicrobial agents than its corresponding copper (II) soaps [24-25].

The trend in inhibition activity of copper (II) sesame soap complexes are found to increase in order:

CSU > CST

Table – 1. Zone of inhibition of two different concentrations of copper (II) sesame soap complexes (50μL/plate) against Staphylococcus aureus.

Compound	PC	C_1 (5×10^4ppm)		C_2(2.5×10^4ppm)		NC
		24hrs	**48hrs**	**24hrs**	**48hrs**	
CST	34mm	8mm	8mm	6mm	6mm	NZI
CSU	34mm	13mm	13mm	12mm	12mm	NZI

PC - Positive Control, NC - Negative Control, C_1 and C_2 - two different concentrations of compounds, NZI - No Zone of Inhibition

Table 2. Descriptive statistics bacterial data for copper (II) sesame soap complexes against Staphylococcus aureus.

Compd.	Conc. (ppm)	Count	Sum	Average	Variance	Std. Deviation	Coefficient variance	Std. Error
CST	5×10^4	3	18.5	6.167	0.143	0.3785	0.0614	0.2185
	2.5×10^4	3	18.2	6.067	0.493	0.7023	0.1158	0.4055
CSU	5×10^4	3	36.1	12.033	0.063	0.2517	0.0209	0.1453
	2.5×10^4	3	36.3	12.100	0.570	0.7550	0.0623	0.4359

Table 3. Anova testing bacterial data for copper (II) sesame soap complexes against Staphylococcus aureus.

Compound	SS	df	MS	F	P-value	F crit	R-Square
CST	0.0150	1	0.0150	0.0470	0.8387	7.7086	0.9526
CSU	0.0067	1	0.0067	0.0210	0.8916	7.7086	0.9900

SS - sum of squares, df - degree of freedom, MS - mean square, F crit - Fcritical, p < F (level of significance)

IV.CONCLUSION

In conclusion, the present work shows relevant biological properties of newly bio-based surfactants from natural edible oil such as sesame, in terms of antimicrobial activities against Staphylococcus aureus. Biological studies demonstrate that the ligand urea and thiourea contains nitrogen, oxygen, and sulphur atoms is responsible to enhance the performance of synthesized complex molecule. The enhanced activity of newly bio-based copper (II) soap complexes can be explained on the basis of chelate formation, the presence of donor atoms such as nitrogen, sulphur and the structural compatibility with molecular nature of the toxic moiety. It has been observed that the antibacterial activity increases with the increase in the concentration of the solution.

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VI. CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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